Visual Flight Control in the Honeybee

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Declaration

The following provides an account of my contribution to the studies presented in this thesis.

Chapter 2: Richard Lamont assisted in the recording and data collection and wrote the software that was used to track bees and generate three-dimensional trajectories.. I designed and ran the experiments and performed all of the analyses. The conclusions are my own.

Chapter 3: I designed the wind tunnel that was used in this study. An apparatus like this has not been described in the literature, so its development required research into small wind tunnel design and extrapolation from fluid mechanics theory. I designed and ran all of the experiments and performed all of the analyses, the conclusions are my own.

Chapter 4: The hypothesis and the supporting mathematical model were developed by Prof. Srinivasan. The mathematical calculations that appear in this thesis were developed from Prof. Srinivasan's hypothesis but they were derived independently and then tested against Prof. Srinivasan's calculations to ensure that they were correct.
I designed and ran all of the experiments and performed all of the analyses.
The conclusions were based on the predictions of the model and therefore, were related to Prof. Srtinivasan's original hypothesis although the discussion represents my own interpretations. The observations and discussion regarding the implications of the changes in the lateral component of flight are my own.

Chapter 5: The active gaze theory for ground height control is my own. I developed the theory that honeybees use optic flow cues generated by regular changes in lateral position to control their ground height and to gain information about the spatial layout of their environment from observations made throughout the course of my Honours and PhD work.

I declare that the results presented in this thesis are my own original work.

Emily Baird

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Abstract

In a single foraging flight, a honeybee may navigate over large distances across open and often turbulent environments, avoid collisions in the cluttered spaces around bushes and trees and make repeated landings on flowers of different shapes, sizes and orientations. The studies described in this thesis investigate the mechanisms of visual ground speed and ground height control in both freely flying and landing honeybees. The findings of this thesis reveal details of the elegant and computationally efficient strategies that honeybees use in order to achieve these extraordinary feats of flight control.

Previous research has shown that honeybees regulate their ground speed by holding the rate of optic flow constant. The efficacy of this simple strategy is highlighted by some of the novel findings presented in this thesis which reveal that honeybees are able to maintain a constant forward speed in both outdoor and indoor flight tunnels, over short and long distances and in the presence of head and tail winds. Detailed analyses of flight trajectories reveal for the first time that honeybees are also able to sustain a constant forward speed despite modulations in the lateral and vertical components of flight.

Little is known about the mechanisms of ground height control in flying insects. The results of this thesis reveal for the first time that honeybees use lateral optic flow cues to regulate ground height. Based on the findings of this thesis, a novel theory of ground height regulation is proposed. The 'active gaze' theory of ground height control proposes that during flight honeybees actively change their lateral position in a consistent manner to extract ground height information from the lateral component of optic flow in the ventral visual field. It is hypothesised that the active gaze strategy of ground height control may also have a more general function that enables honeybees to gain information about the three-dimensional structure of their environment during forward flight.

The findings of this thesis reveal the details of the honeybee's simple, yet elegant strategy for accomplishing smooth landings on a vertical surface. The strategy used for landing on vertical surfaces is an extrapolation of that used for landing on horizontal surfaces. It is hypothesised that the strategy that is described here for facilitating smooth landings on vertical surfaces represents a computationally simple method for ensuring that the speed of approach will be reduced to near zero when contact with a surface is made, regardless of its orientation

The strategies of visual flight control described in this thesis reveal how a simple brain can extract and apply information from the visual scene to control flight. They also suggest novel, biologically-inspired ways, for understanding how these tasks can be achieved in autonomous flying vehicles.

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Chapter 1

General Introduction

The navigational achievements of the honeybee have for centuries fascinated apiarists, researchers and casual observers alike. The interest in honeybee navigation is due not only to their ability to fly up to ten kilometres to a food source and fly the shortest route back, but also to their ability to communicate the location of the food source to other foragers in the hive (for a review, see: von Frisch 1993). These navigational feats are made possible by the honeybee's ability to maintain stable flight. To achieve this, honeybees must be able to control the principal forces of drag and lift by regulating both their ground speed and their ground height in a reliable way. Despite possessing a brain that contains less than one million neurons, honeybees are able to process with extraordinary accuracy all of the complex sensory information that is necessary for achieving stable flight. Honeybees overcome the limitations of their small brain by employing a range of computationally simple techniques to aid flight control and navigation. By studying the mechanisms of ground speed and ground height control in the honeybee, it is possible to gain some insight into how a small and relatively simple brain can achieve the high level of sophistication that is necessary for stable flight and navigation.

Optic flow

For safe and reliable navigation, honeybees require information about their current position, speed and orientation in space and information about the proximity of surfaces in their environment. Investigations into honeybee navigation show that they extract this information, to a large extent, from the pattern of visual motion that is

generated on their retina during flight (for a review see: Srinivasan and Zhang 2004). This pattern of apparent motion is called optic flow. Properties of optic flow, such as the direction and velocity of motion in the visual scene, provide useful cues for detecting course deviations or the proximity of objects in the environment. Honeybees are known to use optic flow information to stabilise flight, estimate the range of objects, negotiate narrow gaps and estimate the distance flown to a food source (Kirchner and Srinivasan 1989; Srinivasan et al 1991; Srinivasan et al 1996; Srinivasan et al 2000a).

To be able to extract information from the pattern of optic flow honeybees and other flying insects must have visual systems that are capable of detecting and measuring motion in the visual scene. Based on the findings of a series of behavioural experiments in flies, Reichardt (1969) proposed a mechanism of motion detection that would enable insects to obtain information from the pattern of optic flow they experience during flight. According to this model, the direction of motion of the scene is extracted by computing the temporal correlation between the signals from neighbouring photoreceptors when there is movement in the visual scene (Reichardt, 1969; Borst and Egelhaaf, 1989).

The validity of the Reichardt model as a representation of the neural underpinnings of the system of motion detection in the insect visual system has been affirmed by the accuracy with which it can be used to predict the strength of some motion-driven behavioural responses. The Reichardt model, and modifications of it are also able to account for the response properties of neurons mediating this behaviour in many species of vertebrates and invertebrates (Egelhaaf and Borst, 1989; Egelhaaf et al., 1989; Hausen, 1993; Krapp and Hengstenberg, 1996; Single et al., 1997).

Rotational optic flow

Flying insects must be able to detect and correct for rotations about the body axes to avoid deviations in flight direction and achieve a stable flight course. When an insect rotates about its vertical axis (yaw) the image of the environment on its retina rotates in the opposite direction. Many insects stabilise their flight course by detecting such

changes in optic flow and performing compensatory gaze adjustments (review: Reichardt 1969; Hengstenberg 1993; Srinivasan and Zhang 2004). This behaviour is known as the optomotor response (Reichardt 1969). The compensatory movements of the optomotor response operate as a reflex course correction and flight stabilisation mechanism (Kaiser 1975). For example, if an insect is flying on a straight path and a gust of wind generates an unintended yaw to the right, the eyes would experience an image shift to the left. The resulting optomotor response would allow the insect to stay on course by generating a compensatory yaw to the left. The optic flow cues that are generated by rotations about the yaw, pitch or roll axes of the insect, known as rotational optic flow cues, thus provide insects with important information about changes in flight direction.

Translational optic flow

Rotational optic flow cues do not contain any information about the distance to surfaces or the translational speed of the viewer with respect to these surfaces (Koenderink 1986). Insects extract translational speed and range information from the visual scene by relying on information generated from translational rather than rotational changes in position. The pattern of optic flow generated during translation radiates from a stationary point, known as the focus of expansion, which is centred in the direction of motion (Gibson 1950). The apparent speed of a point on the image increases with its angular distance from the centre of expansion which produces no apparent image motion. This general pattern of translational optic flow is modified depending on the distances between the viewer and surfaces in the environment. For a constant speed and direction of translation, the angular velocity of a point in the image will increase as the distance between the observer and the surface decreases, i.e. surfaces that are closer generate higher image angular velocities than those that are further away, a phenomenon known as motion parallax.

Information about the absolute distance to surfaces can be obtained from translational optic flow. The apparent angular velocity ($\frac{d\theta}{dt}$) that is generated by a

surface varies with the distance at which it is being viewed (h) according to the following relationship:

$$\frac{d\theta}{dt} = \frac{V}{h} \tag{1}$$

where V is the velocity of the eye with respect to the surface and θ is the angle from the vertical at which the surface is being viewed (e.g. Nakayama and Loomis 1974). If an animal knows how far or how fast it has moved its eyes, it can compute the distance to a surface from the angular velocity that the surface generates on the retina. The first evidence that insects use motion parallax information to estimate the distance to surfaces came from a series of experiments on locusts (Wallace 1959). Prior to jumping across a gap, locusts make side to side translational movements of their head. These head motions, known as 'peering' represent an active gaze strategy that allows the insects to estimate the distance to objects by measuring the changes in optic flow that are generated by these lateral translations in eye position (Wallace 1959; Collett 1978; Sobel 1990; Horridge 1986; Poteser and Kral 1995). Locusts and mantids perform these peering movements when they are not in locomotion. This makes the task of extracting information about the distance to objects a relatively simple one, as the retinal velocities of the fixated object that are generated by the lateral translations of the insect's head can be directly translated into distance information.

Is it possible for flying insects to estimate the distance to the ground by using an active gaze strategy in flight? The optic flow pattern generated in flight is more complicated as, unlike when the body is stationary, it comprises components of axial, vertical and horizontal translation. As a result, the changes in angular velocity produced by peering movements would need to be extracted from the overall pattern of optic flow that is induced by three-dimensional translation. There is evidence that insects such as wasps (Voss and Zeil 1998) and honeybees (Lehrer and Srinivasan 1994) use active gaze strategies to determine the distance to novel objects and to detect the edges of surfaces.

Kern et al (2005) show that motion computation in a blowfly visual interneuron is tuned to make efficient use of the characteristic dynamics of optic flow. The neuron

is able to extract information about the spatial layout of the environment by utilising intervals of stable translation resulting from the saccadic viewing strategy of the fly (Schilstra and van Hateran 1998). Between saccades, the neuron provides information about translatory self motion and thus, implicitly, about the spatial relation of the animal to its surroundings (Boeddeker et al 2005; Kern et al 2005, 2006; van Hateran et al 2005; Karmeier et al 2006). However, it is not known whether flying insects actively change the position of their eyes during free flight to gain information about the distance to nearby surfaces.

Further evidence that flying insects exploit information contained within the pattern of translational image motion during free flight is provided by studies of the navigation behaviours of the honeybee. Due to their small inter-ocular separation, honeybees are unable to use stereoscopic vision to measure the distances to surfaces (Collett and Harkness 1982; Horridge 1986; Srinivasan 1993). Yet honeybees display the remarkable ability to centre themselves when flying through narrow gaps. Despite their lack of stereo vision, this 'centring' behaviour suggests that honeybees are able to determine the distance to the edges of the gap. An investigation into this remarkable behaviour, known as the 'centring response', revealed that honeybees balance the rate of translational optic flow in each eye when flying through narrow spaces (Kirchner and Srinivasan 1989). By balancing the rates of translational optic flow in the two eyes, honeybees can automatically ensure that they are centred between the surfaces of the gap without requiring any absolute distance information. Other investigations have provided further evidence that honeybees determine the relative distance of an object by the speed of the translational optic flow generated on their retinas (Lehrer et al 1988; Srinivasan et al 1989, 1991; Srinivasan and Zhang 2000). The ability to infer range from image motion allows honeybees to avoid collisions by centring themselves in narrow spaces and to detect and avoid obstacles they may encounter whilst flying. This is illustrated by the tendency of honeybees to avoid flying toward rapidly moving objects, a behaviour known as the movement avoidance response (Srinivasan and Lehrer 1984; Srinivasan and Zhang 1997).

Ground speed control

Several investigations have revealed that translational optic flow cues are also important for ground speed control in insects. David (1982) found that *Drosophila,* flying in a wind tunnel with movable patterns on the walls, adjusted their ground speed to keep the velocity of translational image motion on the eye constant. Srinivasan et al (1996) found that honeybees flying through a tapered tunnel decreased their ground speed as the tunnel narrowed and increased their ground speed as it widened. Based on this result, Srinivasan et al concluded that the honeybees were regulating their ground speed to keep the speed of the image of the walls constant. This was tested directly in a further study by Baird et al (2005). The results of this study show that, when flying in a tunnel with moveable patterns on the walls, honeybees reduce their ground speed when the patterns are moved against the direction of flight (generating an increase in apparent image motion) and increase their ground speed when the patterns are moved in the direction of flight (generating a decrease in apparent image motion). The change in ground speed observed in response to the speed and direction of pattern motion on the walls of the tunnel was consistent with the change that would be required if the honeybees were holding constant the axial (front-to-back) component of translational optic flow. This study confirmed the hypothesis of Srinivasan et al (1996) that honeybees regulate their ground speed by holding the rate of translational optic flow constant.

What are the consequences of maintaining the axial component of translational optic flow constant during flight? Because perceived image velocity is related to the distance of the viewer from the substrate, ground speed is adjusted according to the proximity of objects and surfaces in the environment. Although this strategy will not achieve a constant ground speed – the ground speed will depend upon the distances to objects and surfaces in the lateral fields of view – it will ensure that the ground speed is automatically adjusted to suit the flight environment. For example, ground speed would tend to be high when flying in an open field, where distances to surfaces are large and low during flight through dense vegetation, where distances to surfaces would be small. Maintaining a constant image velocity in the eye would ensure that the speed of flight is automatically adjusted to a level that is safe and appropriate to

the environment. This mechanism of ground speed control represents a simple and elegant strategy that automatically regulates flight in a safe and reliable way without requiring any knowledge of ground speed or the absolute distance to surfaces in the environment.

Ground height control

Is ground height regulation important for flying insects? Evidence that ground height regulation is functionally relevant to honeybee behaviour comes from what is known about the mechanisms that underlie the visually driven odometer. Recent studies show that honeybees estimate the distance flown on the journey to a food source by integrating the amount of optic flow that they have experienced during the journey (Esch and Burns 1995, 1996; Srinivasan et al 1996, 1997, 2000a; Esch et al 2001; Si et al 2003). The amount of optic flow that a honeybee experiences on the flight to a food source depends on the distance to surfaces in the environment. The amount of optic flow experienced during flight will increase as ground height decreases: flight at a low ground height will experience more optic flow and thereby estimate a larger distance than flight at a higher ground height (Srinivasan et al 2000a). A honeybee's estimate of the distance flown will therefore depend on the ground height at which it flies. The reliability of the honeybee's odometer, as demonstrated by the precision with which naïve honeybees are able to locate a food source based on the odometric estimate of an experienced forager, thus depends upon their ability to estimate and control their ground height. Indirect evidence from the honeybee's visual odometer therefore provides support for the functional role of ground height control in the honeybee.

What cues do flying insects use to regulate their ground height? Previous studies show that insects flying at different ground heights will fly at different ground speeds (*Drosophila*: David 1982; Moths: Keunen and Baker 1982; Beetles: Fadamiro et al 1998). However, in each of these investigations, the flight paths of the insects were dictated by an odour plume that regulated the height at which the insects flew. In these studies, the insects were using odour cues to regulate ground height. These studies were conducted in the smooth air of the wind tunnel over small distances.

However, in the often turbulent air conditions of the natural environment, odour cues would be unreliable, especially for regulating ground height over large distances. It is therefore unlikely that insects like the honeybee which can fly up to 10 km from the hive in a single foraging trip rely on odour cues to regulate their ground height.

David (1979) provided the first evidence that optic flow cues may play a role in ground height regulation in flying insects. In this study, the effect of optic flow on the ground height of *Drosophila* was tested by moving patterns on the floor of a wind tunnel. When the speed of image motion in the ventral visual field was increased by moving the patterns against the direction of flight, the flies increased their ground height. However, the flies also increased their ground height in response to a decrease in the rate of optic flow the insects experienced in their ventral visual field caused by moving the patterns on the floor of the tunnel in the direction of flight. From these results, David concluded that the increase in ground height that was observed in response to moving the floor patterns in either direction represented a movement avoidance response. This study indicated that the ground height of *Drosophila* is affected by changes in optic flow in the ventral visual field but it did not reveal what cues the insects were using to control their changes in ground height. A recent study by Baird et al (2006) provided the first direct evidence that optic flow cues are important for ground height control in flying insects. The authors found that, when the texture on the floor of a flight tunnel is a longitudinal stripe pattern, honeybees fly lower than when the floor of the tunnel displays a chequerboard pattern. The results of this study reveal that optic flow cues in the ventral visual field play a role in ground height control in the honeybee. Although previous investigations have provided an indication that flying insects use visual cues to control ground height, little is known about how insects use these cues to obtain information about their height above the ground.

Current knowledge about the relationship between visually guided ground speed and ground height control in the honeybee is limited. Previous investigations into the mechanisms of ground speed (Srinivasan et al 1996; Baird et al 2005) and ground height (Baird et al 2006) control in the honeybee are based on studies of flights over short distances (over less than 1 m) in small, indoor tunnels. It is unclear how the

behavioural responses observed under these conditions relate to the behaviour of honeybees flying over large distances in an outdoor environment. The mechanisms of visually guided ground speed and ground height control is investigated in an outdoor setting in Chapter 2. By investigating flight control over large distances in an outdoor environment it is possible, for the first time, to gain some insight into how the behaviours that have been observed in the laboratory environment translate to the flight behaviour of the honeybee in a natural setting.

How would the mechanism of flight control employed by the honeybee respond to wind? To maintain a constant rate of optic flow in the often turbulent air conditions of the natural environment, it would be necessary for honeybees to compensate for the effect of head and tail winds on ground speed. One method of maintaining a constant rate of optic flow in strong head or tail winds would be to restore the apparent angular velocity generated by the ground to its original value by decreasing or increasing ground height. The relationship between ground speed and ground height in wind has not been investigated experimentally. This investigation is carried out in the study described in Chapter 3. This chapter investigates the effect of head and tail winds on the visual regulation of ground speed and ground height of honeybees flying under different conditions of optic flow. This investigation will aid in the understanding of the relationship between the visual mechanisms of ground speed and ground height control and how their response changes in the speed and direction of air flow.

Flight control during landing

To achieve smooth and safe landings, flying insects must be able to regulate their speed in such a way that it is near zero when they make contact with the surface. Few studies have investigated the cues that insects use to control their ground speed when landing. Research into the landing strategies of flying insects has focussed on the cues used to initiate the final step of the landing response – the extension of the insect's legs. These studies show that flies rely on optic flow cues to determine when to extend their legs in anticipation of contact with an object (Goodman 1960; Borst and Bahde 1986, 1988; Wehrhahn et al 1981). Wagner (1982) shows that the onset of

deceleration of flies approaching a spherical target is dependent upon the ratio of retinal velocity and retinal size of the target. While these studies show that flying insects rely on optic flow cues to prepare for landing, they do not reveal how insects regulate their ground speed as they approach a surface in order to produce a smooth touchdown.

Control of ground speed and ground height are crucial to the ability of honeybees to execute smooth landings. In an investigation into how honeybees make grazing landings on horizontal surfaces Srinivasan et al (1996, 2000b) discovered that, as the landing surface is approached, the forward speed and ground height of a honeybee decreases steadily. This deceleration in ground speed is directly proportional to the height of the honeybee above the surface. The authors concluded that honeybees achieve a smooth landing by holding constant the image velocity of the surface. By using image velocity to control flight during landing, ground speed and ground height can be reduced to zero at touchdown without requiring any explicit knowledge of the absolute or moment to moment distance from the surface. Ground height and ground speed regulation through optic flow cues thus provide a simple, yet highly effective technique for the execution of smooth landings on horizontal surfaces.

When a honeybee approaches a horizontal surface, the optic flow it experiences will be dominated by axially-directed image motion. The strategy of holding axial optic flow constant would only produce a smooth touchdown when the optic flow generated by the surface contains image motion in the axial direction. It is not clear what strategies honeybees use to land on surfaces of other orientations, such as inclined or vertical surfaces, which would generate very different patterns of optic flow. Surfaces that are vertically oriented would not necessarily generate any optic flow in the axial direction. Chapter 4 develops a theoretical model of a flight control strategy which regulates ground speed when landing on a vertical surface and test the model predictions with empirical observations of honeybees landing on a vertical surface under different optic flow conditions. This investigation will not only provide insights into how visual information is used to orchestrate landing on a vertical surfaces but also shed light on general strategies that honeybees might use when landing on surfaces of any orientation.

Aims

The control of ground speed and ground height are crucial aspects of stable flight and navigation, yet very little is known or understood about the mechanisms that honeybees use to achieve these behaviours. This thesis investigates the mechanisms of visually guided flight control in the honeybee in free flight and landing. By analysing free flight behaviour during cruising flight and landing in the honeybee, the aim of this study is to discover the strategies that are used by a simple brain to achieve these complex tasks.

The aim of Chapter 2 is to investigate the mechanisms of visually guided ground speed and ground height control in an outdoor setting.

The aim of Chapter 3 is to investigate the relationship between ground speed and ground height in wind.

The aim of Chapter 4 is to develop a theoretical model of a flight control strategy which regulates ground speed when landing on a vertical surface and to test the model predictions with empirical observations of honeybees landing on a vertical surface.

Interest in the flight control strategies of insects has expanded rapidly in recent times as engineers seek to develop computationally simple visual guidance systems for applications in autonomous aerial vehicles. The elegant strategies described in this thesis not only reveal how a simple brain can extract and apply information from the visual scene to control flight. They also suggest novel, biologically-inspired ways, for understanding how these tasks can be achieved in autonomous flying vehicles.

Chapter 2

Visual Flight Control in an Outdoor Setting

Introduction

The ability to control ground speed is fundamental for reliable navigation in the turbulent air conditions of the natural environment. To maintain a constant groundspeed, it is necessary that a flying insect obtain information about its speed relative to the ground. Honeybees regulate their ground speed during flight by holding constant the axial (front-to-back) speed of the image of the environment (Srinivasan et al 1996; Baird et al 2005). Because the apparent speed of the image of the ground will vary with ground height, the ground speed of a honeybee will vary with the height at which it flies. Very little is known about how honeybees obtain ground height information. As a result, the relationship between the mechanisms of ground speed and ground height control in the honeybee is not well understood.

How do honeybees acquire information about their ground height? A recent study by Baird et al (2006) provided the first direct evidence that visual cues play an important role in ground height control in the honeybee. In this study, the ground speed and ground height of honeybees were recorded when the axial optic flow cues (optic flow cues generated by axial translation) on the floor of an experimental tunnel were either strong (chequerboard) or weak (longitudinal stripes). In both cases the walls of the tunnel provided strong optic flow cues in the lateral visual field. The results suggest that honeybees fly faster and lower when axial optic flow cues on the floor are weak, in comparison to when these cues are strong. This finding indicates that honeybees use axial optic flow cues in the ventral visual field to regulate both

their ground speed and their ground height even when strong optic flow cues are present in the lateral visual field. The findings of Baird et al provide the first experimental evidence that honeybees use optic flow cues to regulate their ground height. However, these results do not reveal the nature of the information that honeybees extract from optic flow cues in the ventral visual field and how they use this information to regulate ground height.

What component of optic flow do honeybees use to control their ground height? Based on a series of experiments using a bio-inspired robot, Franceschini et al (2007) proposed that insects could regulate both their ground speed and their ground height by measuring the axial optic flow in different regions of the visual field. According to this hypothesis, insects could regulate their ground speed by measuring axial optic flow cues in the *lateral* visual field and regulate ground height by measuring axial optic flow cues in the *ventral* visual field. This theory has not, as yet, been investigated experimentally with flying insects. Evidence against the hypothesis of insect flight control proposed by Franceschini et al comes from experiments carried out on honeybees. Baird et al (2006) show that changes in ventral optic flow cues affect both the ground speed and the ground height at which honeybees fly. If honeybees were using optic flow cues in the lateral visual field to regulate their ground speed, then the absence of axial optic flow cues from the ventral visual field in these experiments should not have resulted in a change in ground speed. These results suggest that honeybees do not use different regions of the visual field to independently regulate ground speed and ground height. To date, the mechanism by which honeybees use optic flow to estimate and regulate their ground height remains unclear.

Most of what is known about the mechanisms of honeybee flight control is derived from experiments that were conducted in small, experimental tunnels positioned inside a temperature controlled facility (Srinivasan et al 1996; Baird et al 2005, 2006). The experimental conditions under which the flight control behaviours have been observed come from an environment that is quite unlike the natural foraging environment of the honeybee. It is unclear how the behaviours that have been observed under controlled indoor conditions relate to the flight control strategies that

honeybees use when foraging in the outdoors environment. With the development of sophisticated analysis software and modern camera systems it is possible, for the first time, to record in three dimensions, the flight trajectories of honeybees flying in an outdoor experimental tunnel. It has therefore become possible to test whether the behaviours that have been observed in an indoor environment relate to the more variable outdoor environment.

The aim of this study is to investigate the role of ventral optic flow cues in ground speed and ground height control of honeybees flying over large distances in an outdoor environment. This was examined by changing the visual texture on the floor of a large outdoor flight tunnel and observing the effect on the three-dimensional flight trajectories of honeybees flying over a distance of nine metres. Observations of the flight trajectories under these conditions indicate that honeybees may actively induce regular changes in the lateral component of flight to obtain visual information about their ground height. To test whether the observed behaviour could represent a viable strategy for ground height estimation, the relationship between head position and flight direction of honeybees flying in an indoor tunnel is examined.

Methods

Experiment 1: Effect of changes in ventral visual texture on flight control

Experimental apparatus

The experiment was carried out on a levelled section of bushland on the grounds of the Australian National University. The experimental tunnel consisted of two 2.5 m high, 27 m long wooden walls set 2.6 m apart (Figure 2.1). The tunnel was closed at one end with a 2.5 m high, 2.6 m wide wooden panel. The construction of the tunnel was such that all of the walls of the tunnel were suspended 20 cm above the surface of the ground. The cloth that was used to occlude the natural ground texture in two of the experimental conditions was suspended from the bottom of the tunnel walls. By suspending the cloth from the bottom of the tunnel walls it was possible to secure it in such a way that wrinkles (which might provide undesired optic flow cues) were minimised.

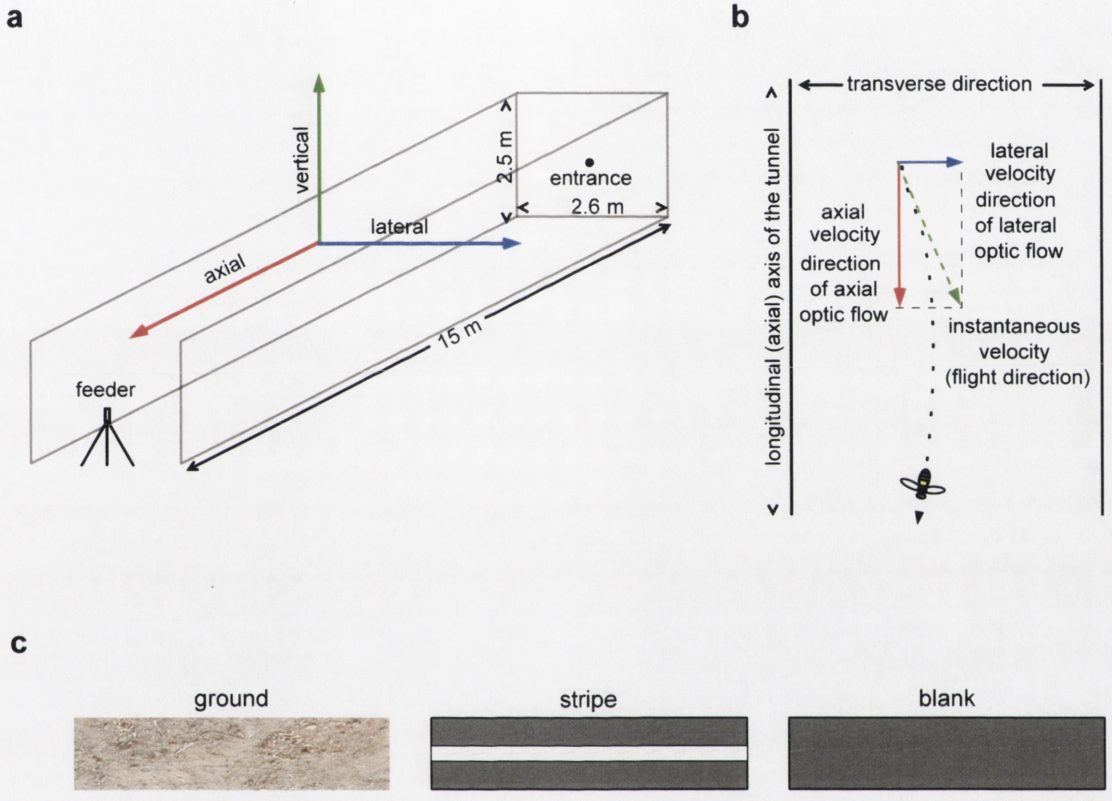

Figure 2.1 Illustration of the outdoor tunnel and visual textures

(a) Illustration of the geometry of the outdoor tunnel and the axes of flight.
(b) Illustration of the direction of flight and optic flow as viewed from above

(c) The visual textures placed on the floor of the outdoor tunnel in Experiment 1

A small beehive was placed on the outer side of the end wall of the tunnel. Honeybees were able to enter the tunnel by means of a plastic tube that ran from the hive through a hole in the end wall which was set 60 cm above the floor of the tunnel. The end wall of the tunnel was painted white which provided a high-contrast background against which honeybees could be identified and digitally tracked (see below). The long walls of the tunnel were painted black in order to limit the visual cues in the lateral visual field of honeybees flying along the tunnel. The top of the tunnel was covered with mesh to prevent unwanted honeybees from entering the tunnel.

Training

Honeybees were trained to fly from the tube in the end wall of the tunnel to a feeder placed in the centre of the tunnel at a distance of 15 m from the end wall. The feeder was placed on a small tripod and set at a height of 60 cm from the floor of the tunnel (to match the height of the entrance hole). The cloth and the stripe textures that were used in the experiment were put in place for at least one day before recording commenced.

Experimental conditions

Flights of honeybees were recorded under three experimental conditions (illustrated in Figure 2.1): ground, stripe and blank. In the 'ground' condition, the natural texture of the ground was visible on the floor of the tunnel, providing the honeybees with strong, natural optic flow cues in the ventral visual field. In the 'stripe' condition, the ground between the hive and the feeder was covered with a dark cloth which had a white stripe 60 cm in diameter running along its length, parallel to the walls of the tunnel. In this condition, axial optic flow cues (optic flow cues generated by axial translation) were minimised, whilst lateral optic flow cues (optic flow cues generated by lateral translation), were retained. In the 'blank' condition, the ground between the hive and the feeder was covered with a dark grey cloth. In this condition, optic flow cues in all directions were minimised. In the following discussions the terms

ground, stripe and blank are used to define the different visual textures used in this experiment.

It is important to note that it would have been instructive to include in this experiment a visual condition where the cloth had white stripes oriented transverse to the longitudinal axis of the tunnel. A transverse stripe pattern would provide axial optic flow cues but no lateral optic flow cues to a honeybee flying along the tunnel. A pattern such as this would have provided a condition with which to compare the effects of removing the lateral component of optic flow with the effects of removing the axial component of optic flow (the longitudinal stripe condition). It was not feasible to include this condition in the present study due to the complexity of laying the stripes on the cloth while it was suspended from the walls of the tunnel. A transverse stripe condition was included in the experiments described in Chapter 4, making redundant the inclusion of such a condition in this study.

Analysis of flight trajectories

Flights between the hive and the feeder were recorded using a stereo camera system that consisted of two synchronised CCD cameras (Pulnix TM-9701s), each equipped with 200 mm lenses. Data from the cameras was captured directly and simultaneously into a computer via a capture card (Euresys Domino Alpha) at a rate of 30 frames per second. The cameras were each positioned at a distance of 50 m from the end wall of the tunnel such that the field of view of each camera converged at a point that was 17 m from the end wall (2 m from the position of the feeder). With this camera arrangement it was possible to reconstruct the real world three-dimensional flight trajectories of honeybees flying over a distance of 15 m between the feeder and the entrance hole. This was done by tracking the position of the honeybees in each camera view and obtaining the pixel coordinates. The system of three-dimensional reconstruction was calibrated and tested by tracking the position of objects in the tunnel that had a known start position and trajectory. The system reliably tracked honeybees from a distance of 50 metres with a calculated positional accuracy of 100 mm in the axial direction, 3 mm in the vertical direction and 3 mm in the lateral direction. At 33 m these reduced to 42 mm, 2 mm and 1 mm for axial,

vertical and lateral axes, respectively. Segments of flights within 3 m of the hive and the feeder were excluded from the analysis in order to leave out any flight manoeuvres that might be related to landing behaviour.

Fourier transform analyses were performed on the lateral velocity values obtained from each individual flight using Matlab (The Mathworks Inc). Observations of the individual power spectra for each flight indicated that, in almost all flights, the lateral oscillations of the honeybees contained a single, dominant frequency. The dominant frequency for each flight was obtained by finding the frequency value with the highest power. These values were then averaged across flights to obtain the mean frequency value for each pattern condition.

The maximum lateral velocity or amplitude of each oscillation within individual flights was identified by locating the values of lateral velocity where the preceding and succeeding values were smaller, indicating a maximum. It was necessary to use a Gaussian filter to remove the noise (caused by tracking inaccuracies) from the lateral velocity data before performing this analysis in order to be able to accurately identify the maximum velocity for each lateral oscillation. The mean of all the maximum lateral velocity values were then calculated for each flight.

Statistical analysis

It was not possible to identify individual honeybees in the recordings due to the limited resolution of the cameras and the large distances involved in this experiment. It is therefore possible that the flight data includes several flights from the same honeybee. All of the statistical analyses were conducted on the presumption that the effects of using repeated flights from the same honeybee did not influence the variation observed in each experimental condition. Detailed analyses from previous experiments (Baird et al 2005, 2006 and the study presented in Chapter 4 of this thesis) have indicated that the variation between flights from the same individual honeybee is similar to the variation between flights from different honeybees. This suggests that repeated flights from the same individual effectively represent random, independent samples within the data set. As a result, standard statistical tests such as

one way analyses of variance (ANOVA) and Student's t-tests were appropriate for all of the analyses. All values presented in the results are given as the mean ± standard deviation.

Experiment 2: Head orientation during flight

Experimental apparatus

The experiments were carried out in an indoor facility at the Australian National University. The experimental tunnel was made of clear Perspex (length: 350 cm; cross-section: 20 cm x 20 cm). Random dot patterns (dot size: 2.5 cm in diameter) were placed on the walls and floor of the tunnel providing strong visual cues in the lateral and ventral visual fields of honeybees flying in the tunnel. The tunnel and the camera that was used to record the flights were covered with a white cloth to allow a clear view of the honeybees' orientation and position whilst recording by minimising reflections from the Perspex roof. This had the effect of minimising optic flow cues in the dorsal region of the visual field by occluding the structures on the roof of the facility.

Analysis of flight trajectories

Honeybees flying to the feeder were recorded over a distance of 20 cm at 400 frames per second using a CMOS camera (Motion Pro 10000 Redlake, Inc.) mounted 180 cm above the central section of the tunnel. The camera was fitted with a macro lens (Nikon 90 mm) that allowed the head and body position of the honeybees flying in the tunnel to be identified. The yaw orientation of the head and body of the honeybees within each flight was determined manually using a program developed in Matlab (The MathWorks, Inc.). The camera system was calibrated by using reference frames from which the known size of objects in the field of view could be measured.

Results

Experiment 1: Effect of changes in ventral visual texture on flight control

Is flight control different when honeybees are flying from the feeder to the hive than when they are flying from the hive to the feeder?

Table 2.1 shows the values for ground speed and ground height for flights from the feeder to the hive and from the hive to the feeder as well as the total value obtained when these results are pooled together for each condition. This data indicates that, within each condition, ground speed and ground height are the same for flights both to and from the feeder. In light of this result, all of the flights from each condition were pooled together for the following analyses.

Table 2.1 The ground speed and ground height of flights in each experimental condition

	Ground speed ($cm.s^{-1}$) mean ± sd				**Ground height (cm) mean ± sd**			
Condition	Feeder to hive	Hive to feeder	Test for difference	Total pooled	Feeder to hive	Hive to feeder	Test for difference	Total pooled
Ground	370 ± 78 n = 33	367 ± 78 n = 11	$t_{43} = 0.13$ p = 0.895	362 ± 71 n = 44	119 ± 17 n = 33	115 ± 16 n = 11	$t_{43} = 0.6$ p = 0.568	119 ± 15 n = 44
Stripe	318 ± 45 n = 41	336 ± 44 n = 16	$t_{57} = 1.3$ p = 0.189	323 ± 45 n = 57	113 ± 19 n = 41	113 ± 25 n = 16	$t_{57} = 0.1$ p = 0.917	113 ± 20 n = 57
Blank	302 ± 48 n = 92	304 ± 48 n = 28	$t_{119} = 0.2$ p = 0.828	302 ± 48 n = 120	127 ± 15 n = 92	124 ± 27 n = 28	$t_{119} = 0.4$ p = 0.717	127 ± 21 n = 120

Effect of changes in ventral visual texture on axial flight velocity

The dependence between axial ground speed (i.e. speed in the direction aligned with the tunnel's longitudinal axis) and ventral visual texture is shown in Figure 2.2a. Ground speed is greatest in the ground condition but, in comparison, is significantly reduced in the stripe ($t_{100} = 3.2$, $p = 0.0018$) and blank ($t_{162} = 5.2$, $p < 0.0001$) conditions. Ground speed is also significantly lower in the blank condition in comparison to the stripe condition ($t_{176} = 2.8$, $p = 0.0059$). These results indicate that the ground speed of honeybees changes when optic flow cues in the ventral visual field are removed.

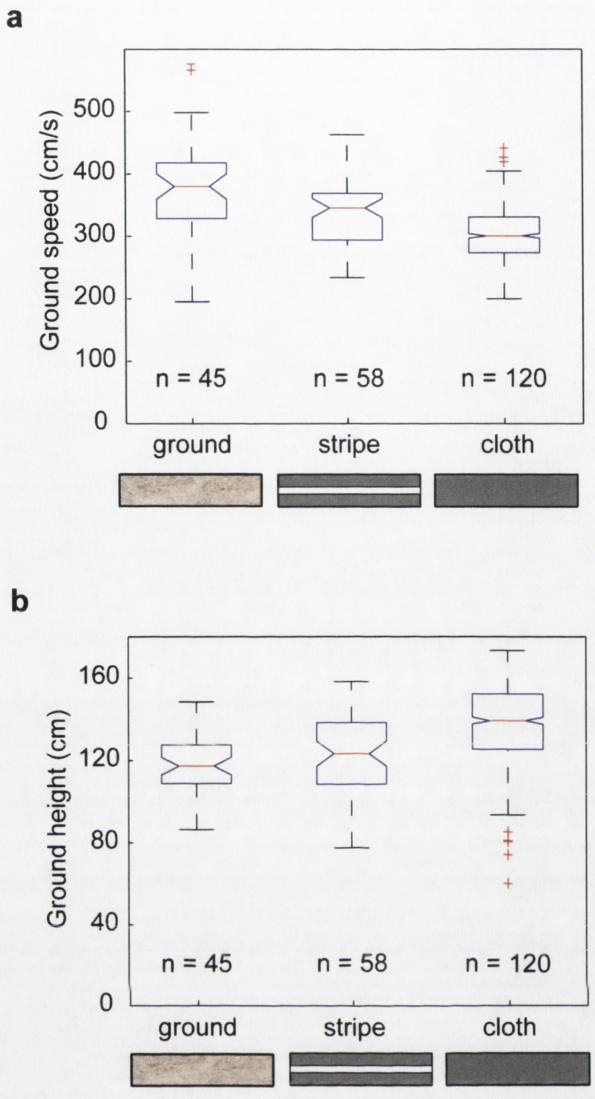

Figure 2.2 Effect of ventral visual texture on ground speed and ground height

(a) Effect of ventral visual texture on ground speed (axial velocity).
(b) Effect of ventral visual texture on ground height

Boxes indicate the distance between the lower and upper quartile values, red lines indicate the median value and whiskers indicate the extent of the rest of the data. Red crosses indicate points whose values exceed 1.5 times the interquartile range. Notches represent the 5% confidence of the median value: boxes whose notches overlap indicate that the medians of the two groups differ at the 5% confidence level.

Effect of changes in ventral visual texture on ground height

The relationship between ground height and ventral visual texture is shown in Figure 2.2b. The mean ground height at which honeybees fly is lowest in the ground condition but is not significantly different from the mean ground height in the stripe condition ($t_{100} = 1.52$, $p = 0.1350$). Honeybees fly at a significantly higher ground height in the blank condition than in both the ground ($t_{162} = 2.53$, $p = 0.0140$) and the stripe ($t_{176} = 4.01$, $p = 0.0001$) conditions. These results indicate that the ground height of honeybees is affected when the optic flow cues on the floor of the tunnel are removed but is unaffected when lateral optic flow cues are present.

Observations of the flight trajectories

Figure 2.3 shows examples of flight trajectories from each experimental condition, which illustrate some very interesting observations. In almost all flights, the relationship between axial position and time is linear, indicating that ground speed along this axis, i.e. axial velocity, is kept constant (Figure 2.3: a(i), b(i) and c(i)). The lateral position of the honeybees oscillated from side to side with relative constancy (Figure 2.3: a(ii), b(ii) and c(ii)). The regularity with which the position along this axis oscillates becomes clear when the velocity of these lateral translations is plotted against time (Figure 2.3: aiii, biii and ciii). A Fourier analysis (Figure 2.3: a(iv), b(iv) and c(iv)) reveals that the changes in lateral velocity contain a single dominant frequency component. The regular, near sinusoidal fluctuations in lateral velocity were observed in most flights across all of the experimental conditions. The ground height, i.e. vertical position, of the honeybees is not always held constant but, unlike the changes in lateral translation, the deviations did not oscillate in a regular pattern (Figure 2.3: a(i), b(v) and c(v)). The relationship between vertical velocity and time contains some small oscillations but they are not as consistent as those observed along the lateral axis and they are not present in all flights (Figure 2.3: a(vi), b(vi) and c(vi)).

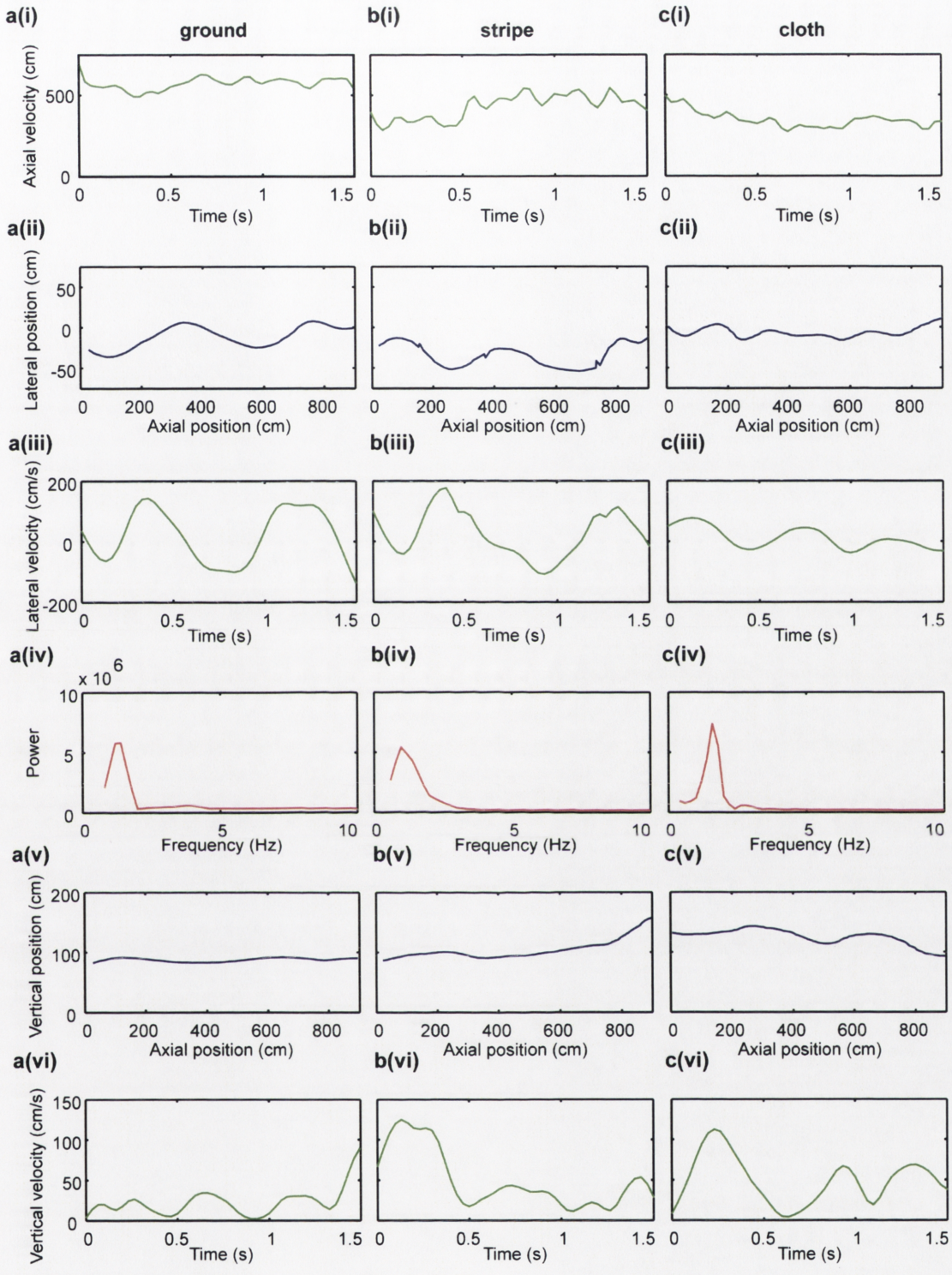

Figure 2.3 Examples of flight trajectories from each of the three experimental conditions

(a) ground condition, (b) stripe condition and (c) blank condition
Roman numerals i – v indicate axial position over distance (i), lateral position over distance (ii), lateral velocity over time (iii), vertical position over distance (iv) and vertical velocity over time (v).

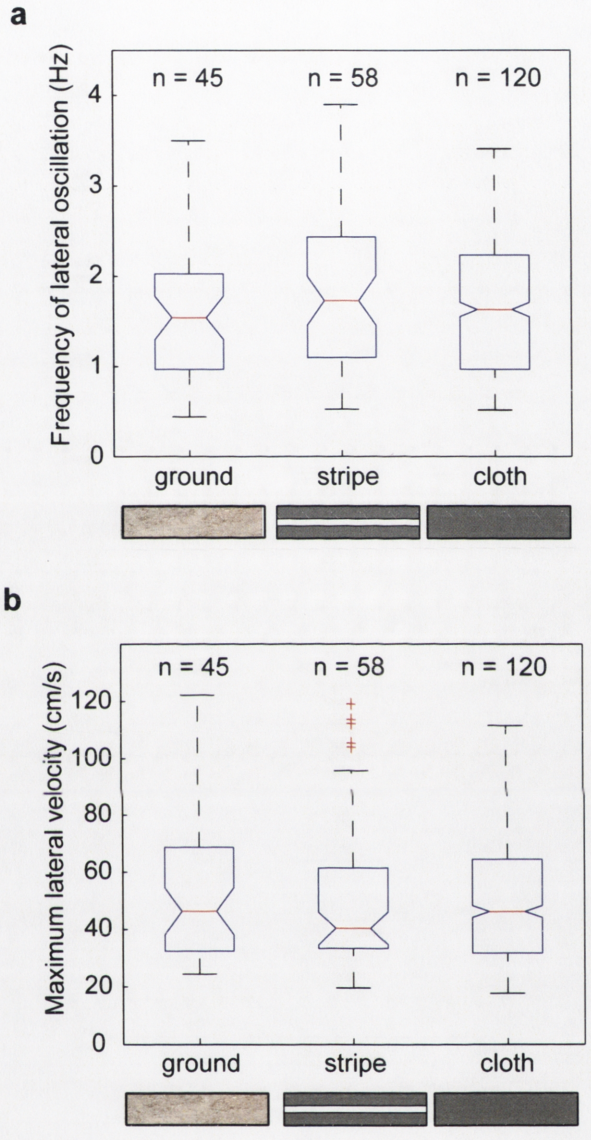

Figure 2.4 Effect of visual texture on the properties of lateral oscillations

(a) Dominant frequency components
(b) Mean maximum lateral velocity (amplitude of lateral oscillations)

Details of the boxplots are described in Figure 2.2.

Effect of visual texture on the properties of lateral oscillations

To test whether the changes in lateral velocity that were observed in each flight occurred at a constant frequency (i.e. contained a dominant frequency component), as the data from individual flights suggested, a Fourier analysis was used. The results of this analysis are shown in Figure 2.4a. Interestingly, there is no effect of visual texture on the dominant frequency component of lateral oscillations ($F_2 = 1.86$, $p = 0.1567$). The frequency of lateral oscillation is 1.65 Hz in the ground condition, 1.71 Hz in the stripe condition and 1.64 Hz in the blank condition. In contrast, no dominant frequency components were evident in either the axial or the vertical components of flight. The results of an analysis on the mean maximum lateral velocity values in each condition are shown in Figure 2.4b. This data indicates that the maximum lateral velocity is not affected by changes in visual texture ($F_2 = 0.37$, $p = 0.6900$). The mean maximum lateral velocity is 57 ± 36 $cm.s^{-1}$ in the ground condition, 53 ± 29 $cm.s^{-1}$ in the stripe condition and 54 ± 27 $cm.s^{-1}$ in the cloth condition. This data indicates that the maximum lateral velocity is not affected by changes in visual texture. This result further supports the findings of the frequency analysis by showing that neither the frequency, nor the maximum velocity components of the lateral oscillations were affected by changes in the visual texture. The results from these analyses suggest that the oscillations that were observed in the lateral component of flight were occurring in a systematic, near sinusoidal way, regardless of the changes in texture in the ventral visual field.

Experiment 2: Head orientation during flight

Based on the analysis of the flight trajectories in the previous experiment, it is hypothesised that honeybees actively change the lateral component of flight to obtain ground height information from the lateral optic flow cues that are generated in the ventral visual field. To test the validity of this hypothesis, it is necessary to investigate whether the visual field remains oriented in the axial direction of flight and whether it is rotated during these changes in lateral position (the reasons for this

are described in detail in the discussion). This can be achieved by examining the orientation of the head (and hence the eyes) with respect to the changes in flight direction (the instantaneous of forward motion) and the axial direction of flight (the mean direction of flight). It is not possible to gain this information from the recordings made in the first experiment due to the limited resolution of the cameras and the large distances over which the flights were filmed. To overcome this, we conducted a second experiment in which flights were recorded at high resolution, permitting an analysis of the honeybees' head orientation during changes in flight direction.

Figure 2.5: a(i)-d(i) shows example trajectories of flights in the small, indoor tunnel. Interestingly, when flying in the small tunnel, honeybees performed lateral translations that were similar to those observed in the outdoor tunnel, although the frequency of oscillations in lateral velocity occurred at a higher rate (~ 4 - 5 Hz). Figure 2.5: a(ii)-d(ii) shows a comparison between the head orientation and flight direction for each of the trajectories shown in Figure 2.5: ai-di. An analysis of head orientation during changes in flight direction reveals that, for a significant proportion (70 ± 19 %) of the total duration of each recording, the head does not follow the changes in flight direction but instead remains oriented in the axial direction of flight. In all but two out of the 20 flights that were analysed in this experiment, the angle of head orientation changed during the flight. When these changes in head orientation, or saccades were made, they were performed rapidly: the head is held at a constant orientation for 86 ± 9 % of the duration of the flight. The results from this experiment indicate that honeybees stabilise their heads against changes in the direction of flight that are induced by lateral translations. Because the gaze direction of the honeybees is held constant against the changes in flight direction, the lateral translations that the honeybees perform during flight would serve to generate lateral optic flow cues in the ventral visual field.

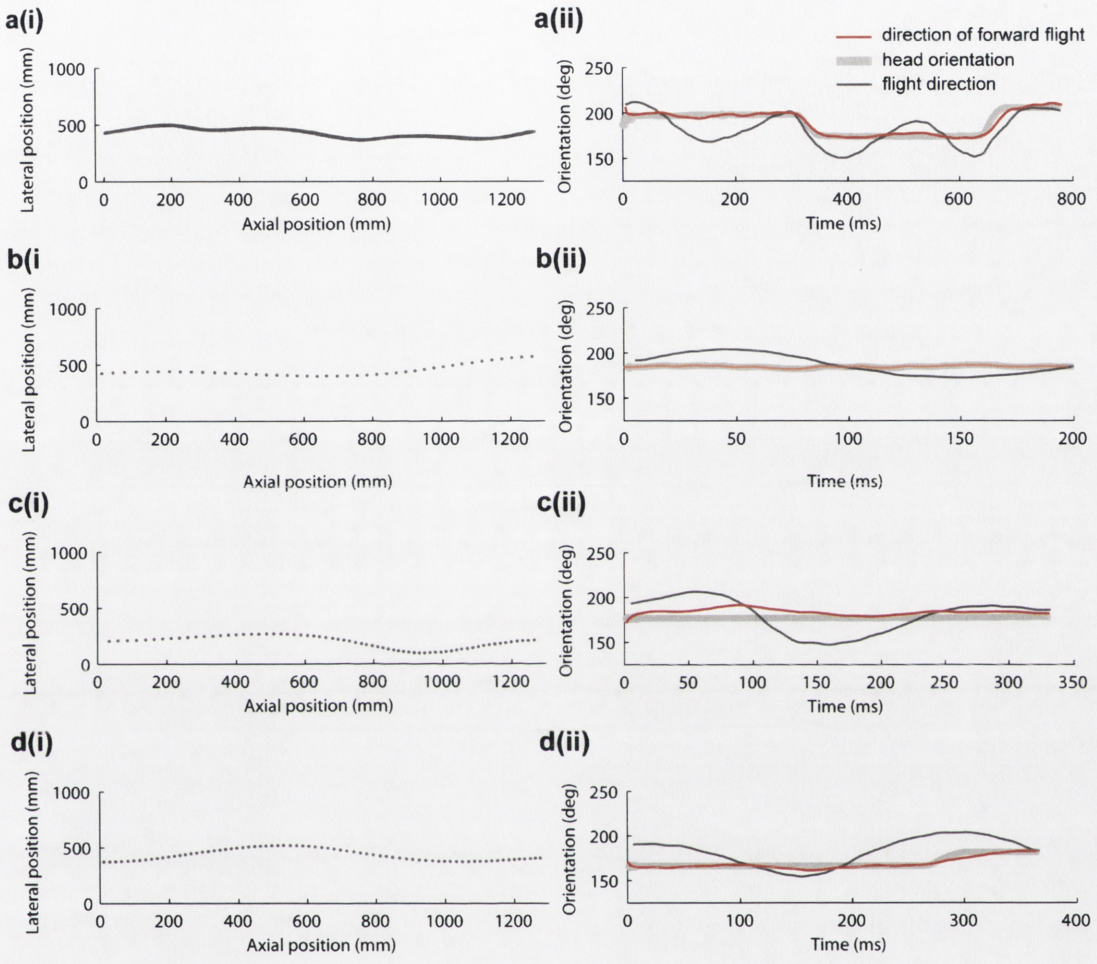

Figure 2.5 Examples of flight trajectories recorded at high resolution

a(i) – d(i): Flight trajectories of honeybees flying in an indoor tunnel. Dots represent the position of the honeybee at every 0.0025 sec time step.
a(ii) – d(ii): Black lines represent the change in the orientation of flight direction, grey lines represent the head orientation and red lines represent the mean direction of flight.

Discussion

The findings of this study suggest that honeybees rely on different components of optic flow to differentially regulate ground speed and ground height. The results provide the first evidence that lateral optic flow cues are important for ground height control in a flying insect. This study also presents, for the first time, detailed three-dimensional information about the flight trajectories of honeybees over large distances in an outdoor environment. During flight, honeybees make regular changes in lateral position while keeping axial ground speed constant. These near sinusoidal lateral oscillations appear to be unaffected by changes in the ventral visual texture. A detailed analysis of head orientation during flight reveals that, whilst performing these lateral translations, honeybees stabilise their head against the subsequent changes in flight direction. The results of this study suggest that honeybees use lateral optic flow cues in the ventral visual field to regulate ground height. Based on these findings, a novel mechanism for ground height estimation is proposed, whereby honeybees actively induce changes in their lateral position to generate lateral optic flow cues in the ventral visual field from which information about ground height is independently obtained.

The role of ventral optic flow cues in the regulation of flight

Honeybees appear to use different components of optic flow in the ventral visual field to regulate different aspects of their flight. To control ground speed, honeybees extract information from the axial component of optic flow, whereas ground height appears to be regulated using information extracted from the lateral component of optic flow in the ventral visual field. When axial optic flow cues in the ventral visual field are minimised, i.e. in the blank and stripe conditions, honeybees fly slower in comparison to flights over the natural texture of the ground, which provides optic flow cues in all directions. This result is consistent with previous studies which have shown that honeybees regulate their ground speed by keeping the rate of axial optic flow constant (Baird et al 2005, 2006). In fact, the axial angular velocity that the honeybees were experiencing in the ground condition (174 $deg.s^{-1}$) is similar to the

angular velocities that were experienced by honeybees flying in an indoor tunnel: ~200 deg.s^{-1} (Baird et al 2005). When axial optic flow cues are minimal however, honeybees are no longer able to regulate ground speed in the same way as when these cues are present. This is evidenced by the decrease in the value of angular velocity of 137 deg.s^{-1} that the honeybees experience in the blank condition (assuming that they experience axial optic flow cues in this condition). Interestingly, the results indicate that ground height is not affected when *axial* optic flow cues are minimised (ground and stripe conditions), but is reduced when *lateral* optic flow cues are minimised (blank condition). These results provide the first evidence that ground height regulation in the honeybee is mediated by lateral optic flow cues. This result conflicts with the hypothesis of Franceschini et al (2007) which proposes that flying insects use axial optic flow cues in the ventral visual field to regulate their ground height. In contrast, the results of the present study indicate that honeybees use axial optic flow cues to regulate their ground speed and lateral optic flow cues to regulate their ground height. Overall, the findings of this experiment imply the existence of parallel pathways of information processing in the honeybee visual system that extract information from the axial and lateral components of optic flow to independently regulate ground speed and ground height.

In the present study, the ground speed of honeybees in the stripe condition is reduced in comparison to the ground condition. However, previous studies have shown that the presence of longitudinal stripes in either the lateral or ventral visual field of honeybees flying in an indoor tunnel caused an increase, rather than a decrease in ground speed when compared to visual textures that provided axial optic flow information (Baird et al 2005; 2006). It is unclear why there is a difference in the effect of longitudinal stripes on ground speed between the indoor and outdoor tunnels. One major difference in the visual environments presented in these two experiments is that the visual textures that were used in the indoor experiments all contained high contrast features in the lateral visual field (i.e. on the walls of the experimental tunnel) whereas in the outdoor tunnel, optic flow cues in the lateral visual field were minimised. The effect of longitudinal stripes in the ventral visual field – in the absence of optic flow cues in the lateral visual field – in an indoor environment has not been tested. It is possible that the lack of lateral optic flow

cues in the outdoor tunnel prevented the honeybees from being able to gauge their distance to surfaces in either the lateral or ventral visual field, causing them to resort to a 'safe' flight strategy – i.e. slowing down. In fact, in the blank condition where optic flow cues in both the lateral and ventral regions of the visual field are minimised, honeybees fly slower and higher. This would be the safest strategy for collision avoidance if an accurate estimate of the distance to surfaces in the environment cannot be made.

Gaze orientation during lateral translation

The results from the first experiment indicate that honeybees use lateral optic flow cues in the ventral visual field. This result, in combination with the observation that the lateral component of flight oscillates in a regular fashion, suggests that honeybees may use these changes in lateral position to generate lateral optic flow cues in the ventral visual field. These lateral optic flow cues, in turn, could be used to provide ground height information as changes in the speed of lateral image motion would indicate changes in ground height. To generate lateral optic flow cues in the ventral visual field, it is necessary that the gaze direction of the honeybee does not follow the changes in flight direction that are generated by changes in lateral position. If the gaze direction follows the change in the direction of flight, the optic flow profile would be dominated by motion in the axial direction. As a result, changes in lateral position would not generate strong lateral optic flow cues in the ventral visual field. If on the other hand, the direction of gaze remains oriented with the axial component of flight (i.e. in the mean direction of flight) then the lateral oscillations in position would generate lateral optic flow cues in the ventral visual field. To determine whether the changes in lateral position generate lateral optic flow cues it is therefore necessary to investigate the relationship between head orientation and flight direction.

Our high temporal and spatial resolution analysis of honeybees flying in the indoor tunnel reveals that head orientation is largely stabilised against the yaw rotations of the body that occur during lateral translation. This gaze stabilisation can serve to minimise rotational distortions in the pattern of image motion generated during

lateral translation, allowing information about distance to the ground to be readily extracted from the lateral component of optic flow. This result is consistent with observations in flies which have shown that these insects stabilise their gaze orientation for a large proportion of time during flight (Schilstra et al 1998; van Hateren et al 1999) and also when walking (Blaj and van Hateran 2004). Head stabilisation against rotation of the type that has been observed here in honeybees has also been described in flies. Between body saccades, flies stabilise their heads (and therefore their eyes) against rotation; when changes in head orientation are made, they occur in rapid, saccadic movements (van Hateren and Schilstra 1999). In the present study, when the honeybees changed their gaze orientation, these changes were brought about by a rapid, saccadic movement. These movements occupied a small proportion of the duration of flight. By making changes in head orientation rapidly, honeybees minimise the effect of the resultant yaw rotation on the pattern of translational optic flow. In this experiment, we could only quantify the yaw orientation of the honeybees' heads during flight. However, recent experiments show that honeybees also stabilise their head orientation against roll rotations during lateral translations (Boeddeker and Hemmi, in submission). Furthermore, Kern et al (2005) found that a motion-sensitive neuron in the fly visual system is capable of extracting information about the spatial layout of the environment from the translational optic flow information that is experienced during such periods of head stabilisation. By minimising the time over which prominent rotational optic flow is generated and utilizing a saccadic viewing strategy, insects can segregate the component of optic flow resulting from rotational movements from the overall pattern of optic flow resulting from translational movements (Schilstra and van Hateren 1998). This can then help to detect disturbances to the intended flight path (Collett 1980), and to gauge the relative distance to objects from discontinuities in the translational optic flow field (Blaj and van Hateren 2004; Land and Collett 1997; Schilstra et al 1998; Srinivasan 1993; van Hateren et al 1999).

Although it has been postulated that flying insects could use periods of head stabilisation during translation to acquire information about the spatial structure of their environment (Kern et al 2005), the mechanism that serves to stabilise the head against rotation is not well understood. Some potential mechanisms for head

stabilisation during flight have been described in dragonflies and flies. In dragonflies, it has been shown that visual stabilisation is aided, at least in part, by the occeli (Goodman 1965; Stange 1981). In flies, the halteres provide information about body rotation, which could aid the insect in stabilising the head and minimising unwanted rotation in the visual field (Nalbach and Hengstenberg 1994; Sherman and Dickinson 2004). However, the mechanism that would mediate head stabilisation of the type that has been observed here in the honeybee is yet to be identified and understood.

Active gaze: a novel mechanism of visually mediated ground height control

By actively changing their lateral position in a regular and non-visually mediated way, it is hypothesised that honeybees regulate their ground height by measuring changes in the lateral component of optic flow in the ventral visual field. When flying over large distances in an outdoor flight tunnel, honeybees make regular oscillations in their lateral position whilst maintaining a constant forward speed. Honeybees do not appear to use visual cues to regulate these changes in lateral position as the frequency and amplitude of the changes in lateral velocity are unaffected by changes in the visual texture on the floor of the tunnel. Whilst changing their lateral position, honeybees stabilise their heads in the axial direction of flight and minimise the effect of rotation in the visual scene by changing head orientation in a fast saccadic movement. Together, these results indicate that honeybees may use the lateral optic flow cues that are generated by changes in lateral position to gain information about their distance to the ground. This is because the speed of lateral image motion will vary inversely with distance from the ground irrespective of the honeybee's axial speed. The findings of this study lend support to the hypothesis that honeybees actively change their lateral position in a consistent manner to extract ground height information from the lateral component of optic flow in the ventral visual field.

Chapter 3

Visual Flight Control in Wind

Introduction

Flying insects must be able to compensate for changes in wind speed and direction to navigate safely and reliably within their environment. Studies of a number of insect species have shown that flying insects use visual information to regulate their airspeed when flying in head winds (Mosquitos: Kennedy 1940; Moths: Kennedy and Marsh 1974; Keunen and Baker 1982; Priess 1987; *Drosophila*: David, 1979; Aphids: Kennedy and Thomas 1974; Locusts: Baker 1979; Honeybees: Barron and Srinivasan 2006). David (1982) demonstrated that *Drosophila* compensate for head winds by increasing their airspeed so as to maintain a constant ground speed. By manipulating the rate of image motion that the flies experienced when flying in head winds, David showed that *Drosophila* maintain a constant ground speed by holding the rate of axial (front-to-back) optic flow constant. This finding is supported by the results of more recent investigations which revealed that, in the absence of wind, honeybees regulate their ground speed by holding constant the rate of axial optic flow on the retina (Srinivasan et al 1996; Baird et al 2005).

By holding constant the rate of axial optic flow, insects are able to maintain a constant ground speed in head winds. A predicted consequence of this mechanism of ground speed control is that, when the insect can no longer produce enough thrust to compensate for a head wind, it will maintain the preferred rate of optic flow by

reducing ground height. When flying closer to the ground, the rate of optic flow on the retina will increase and, as a result, the preferred rate of optic flow can be restored. A similar response might be predicted for insects flying in tail winds, except that the insect would maintain the preferred rate of optic flow by increasing its ground height and thereby reducing the apparent rate of optic flow.

Do insects change their ground height when flying in winds? Observations made during field studies suggest that honeybees (Wenner 1963) and bumblebees (Riley et al 1999) fly lower in head winds and higher in tail winds – as predicted by the mechanism of ground speed control. These observations support the hypothesis that, to maintain a constant rate of optic flow, insects reduce their ground height when flying in head winds and increase their ground height in tail winds. Little is known or understood about the relationship between ground speed and ground height control and how it is affected by wind.

The aim of this study is to rigorously test the hypothesis that honeybees maintain a constant rate of optic flow in strong winds by adjusting the ground height at which they fly. The properties of the visual mechanism that mediates ground speed and ground height control are examined by investigating the effect of different visual textures on ground speed and ground height of honeybees flying in both head and tail winds.

Methods

Training

The experiments were carried out in an All Weather Bee Flight Facility at the Australian National University's Research School of Biological Sciences. The temperature inside the facility was maintained at 24 ± 5 °C during the day. A beehive mounted on the wall of the facility supplied the honeybees (*Apis mellifera* L.) used in the experiments. For each experiment, up to 15 honeybees were trained to fly to a feeder containing sugar solution placed at the end of the test section nearest the fan. Each honeybee was marked with paint for identification of individuals.

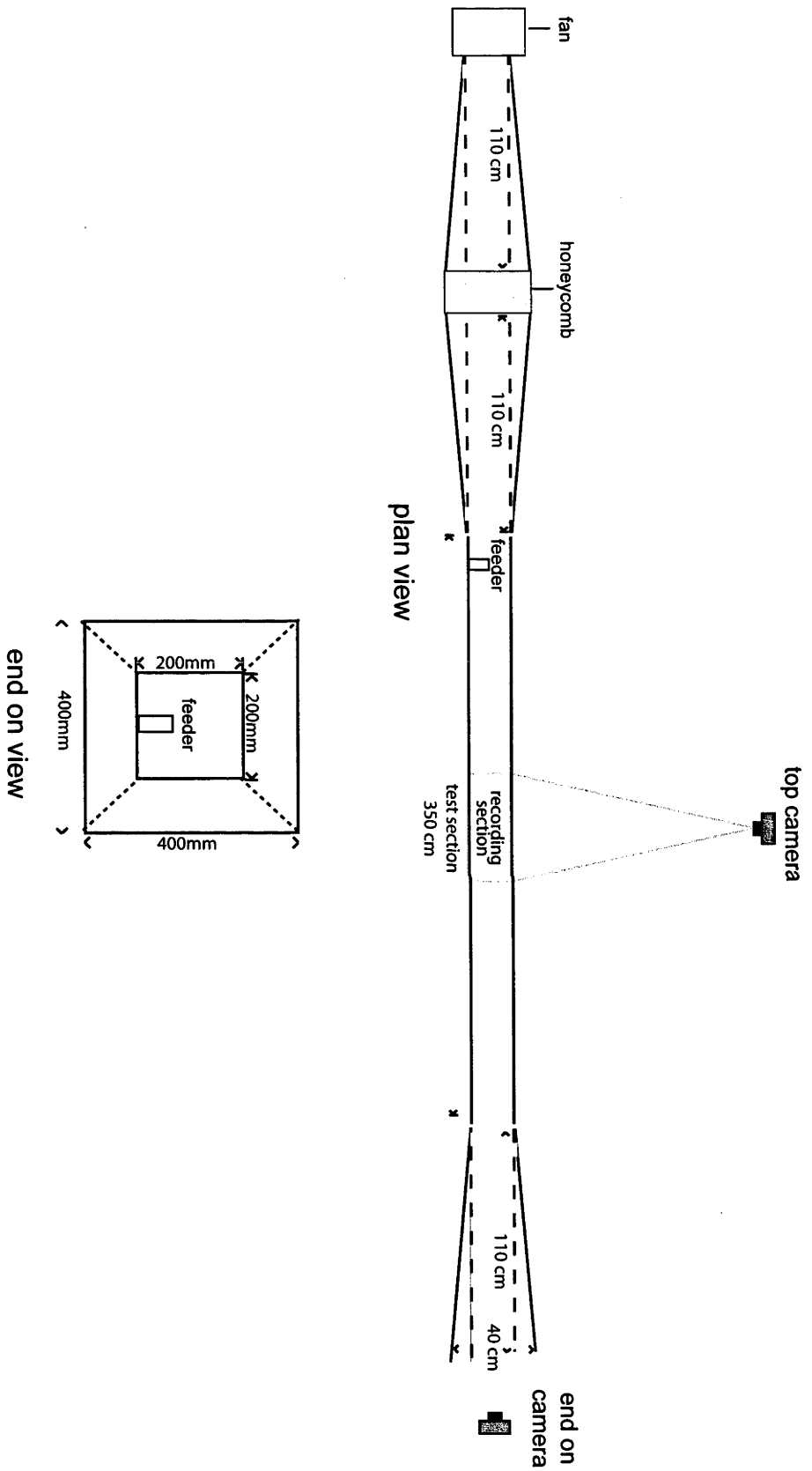

Figure 3.1 Illustration of the wind tunnel set-up with dimensions

Experimental Apparatus

A diagram of the experimental apparatus is shown in Figure 3.1. The experiments were conducted in a rectangular wind tunnel that was specifically designed for these experiments. The body of the tunnel is constructed of clear Perspex. The tunnel has three main sections: an entrance section, a test section, and a settling chamber. The entrance section is 1.1 m in length with a cross-section tapering from 40 x 40 cm at the open end to 20 x 20 cm where it contracts to the test section. The test section of the tunnel was 3.5 m in length and had a uniform cross-section of 20 x 20 cm. An opening at the fan-end of the test section allowed access to the tunnel which was required for the placement of the sugar-water feeder. The end of the test section was attached to a settling chamber which consisted of three component sections. The first section was a 1.1 m long chamber with a cross section that expanded from 20 x 20 cm to 40 x 40 cm. This section attached to the second, honeycomb section that consisted of an array of plastic PVC tubes (each 1.5 cm in diameter) which had a 40 x 40 cm cross section and a width of 15 cm. The honeycomb section of the tunnel was attached to a third section that was 1.1 m long with a cross section that contracted from 40 x 40 cm to 20 x 20 cm, where it connected to the fan. The purpose of the settling chamber was to reduce the turbulence created by the rotation of the fan blades and to ensure that the air flow in the test section was as smooth as possible.

The fan consisted of a vertically oriented array of 10 blades which could be positioned such that rotation in either direction would produce opposite directions of air flow in the test section of the tunnel – that is, head and tail winds. The fan was operated by a variable voltage DC motor that could produce winds up to 10 $m.s^{-1}$ in either direction in the test section of the tunnel. Wind speeds in the test section of the tunnel were calculated using both a hot-wire anemometer and a fan anemometer. The values of wind speed were taken at a number of positions at the fan-end of the test section and were then calibrated with the voltage settings of the fan.

The tunnel and the camera that was positioned above the tunnel were covered with a white cloth to allow a clear view of the honeybees' orientation and position whilst recording while minimising reflections from the Perspex roof. This had the effect of minimising optic flow cues in the dorsal visual field by occluding the structures on the roof of the facility.

Experimental conditions

Four experiments were conducted to investigate the effect of wind on flight control under different visual conditions. The four different visual textures used in this study are shown in Figure 3.2. In each experiment patterns were laid on the floor and walls of the tunnel. The patterns were created by attaching strips of red electrical tape (1.8 cm in width) to 3.5m long strips of white laminated paper. The strips of electrical tape were distributed evenly along either the length or width of the paper (depending on the pattern). Red tape was used in these experiments because it had a lower contrast than black tape (which would provide higher contrast optic flow cues) in the recorded images. The use of red instead of black patterns facilitated the automated tracking process by making it easier to locate the honeybee against the dark regions of the pattern in the recorded images. As honeybees do not have red-sensitive photoreceptors, the red colour would be perceived as a high-contrast dark shade of grey.

In each experiment, flights of honeybees were recorded in three conditions: still air, head winds and tail winds of 1 $m.s^{-1}$ or 2 $m.s^{-1}$. The wind speeds used in these experiments were limited by the maximum tail wind in which the honeybees would enter and fly in the tunnel. Preliminary experiments revealed that, although honeybees would fly to the feeder in head winds of up to 6 $m.s^{-1}$, they would not enter or fly in the tunnel when the tail winds exceeded 2.5 $m.s^{-1}$. At tail winds above this value, honeybees that entered the tunnel would either land on the floor or rapidly orient themselves against the direction of air flow and thus away from the direction of the feeder.

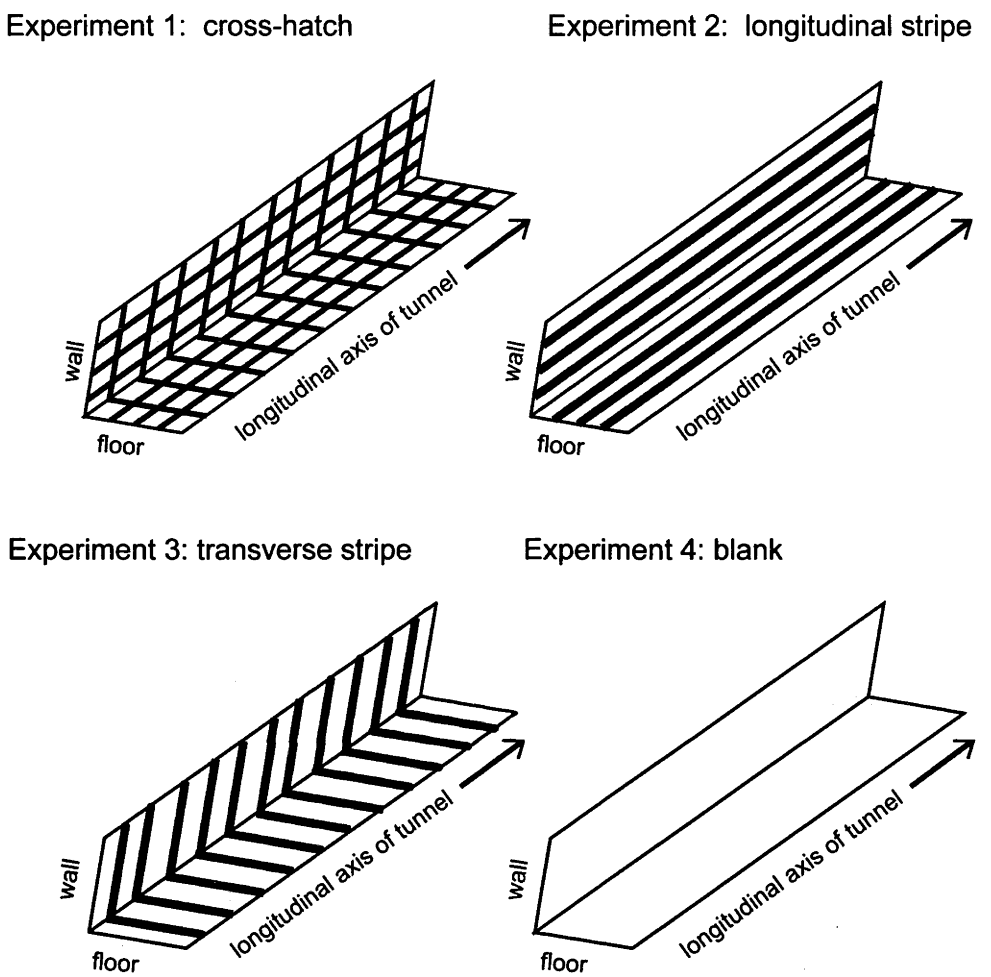

Figure 3.2 The visual textures used in each experiment

A representation of the visual textures in each experiment and their orientation as they would appear on the walls and floor of the experimental tunnel.
Experiment 1: cross-hatch pattern provides axial and lateral optic flow cues

Experiment 2: longitudinal stripes provide lateral optic flow cues in the absence of axial optic flow cues

Experiment 3: transverse stripes provide strong axial optic flow cues in the absence of lateral optic flow cues

Experiment 4: blank provides no optic flow cues.

In each experiment, the pattern was placed in the tunnel two days prior to testing. The honeybees were only exposed to the head or tail winds during the testing periods which lasted for 30 minutes. Between testing periods, the honeybees were allowed to visit the feeder in still air for at least 30 minutes before the commencement of the next testing period. Each experiment was conducted over three days with each wind condition being presented in a randomised order on each day.

Experiment 1: Effect of wind on flight control in the presence of lateral and axial optic flow cues

The effect of wind on ground speed and ground height control was investigated in the presence of strong lateral and axial optic flow cues. In this context, the term 'lateral optic flow cues' refers to optic flow cues that would be generated by lateral translation (i.e. translation along the transverse axis of the tunnel); the term 'axial optic flow cues' refers to optic flow cues that would be generated by axial translation (i.e. translation along the longitudinal axis of the tunnel). The pattern used in this experiment consisted of 1.8 cm thick stripes oriented both along the transverse and longitudinal axes of the tunnel to create a cross-hatch pattern. The stripes in this pattern were distributed with a 2.5 cm separation between the longitudinal stripes and 4 cm separation between the transverse stripes.

Experiment 2: Effect of wind on flight control in the absence of lateral optic flow cues

The effect of wind on ground speed and ground height control was investigated when lateral optic flow cues were removed from the visual texture. The pattern used in this experiment consisted of 1.8 cm wide stripes oriented along the lateral axis of the tunnel. The stripes were distributed evenly with an edge-to-edge separation of 4 cm. The transverse orientation of the stripes would generate strong axial optic flow cues for a honeybee flying to the feeder, whilst lateral optic flow cues would be weak.

Experiment 3: Effect of wind on flight control in the absence of axial optic flow cues

The effect of wind on ground speed and ground height control was investigated when axial optic flow cues were removed from the visual texture. The pattern used in this experiment consisted of 1.8 cm wide stripes oriented along the longitudinal axis of the tunnel. The stripes were distributed evenly with an edge-to-edge separation of 2.7 cm. The longitudinal orientation of the stripes would generate strong lateral optic flow cues for a honeybee flying to the feeder, whilst axial optic flow cues would be weak.

Experiment 4: Effect of wind on flight control in the absence of optic flow cues

The effect of wind on ground speed and ground height control was investigated when all strong optic flow cues were removed from the visual environment. The walls and floor of the tunnel in this experiment were white with minimal features. In this case, the honeybees would not experience strong optic flow cues when flying in the tunnel.

Effect of optic flow on lateral oscillation

The results of Chapter 2 reveal that, when flying in an outdoor tunnel, the lateral component of flight oscillated in a systematic way and does not appear to be affected by visual texture. To investigate whether lateral oscillations observed in this study were affected by visual texture, three analyses were conducted on the lateral component of flight and compared across Experiments 1, 2 and 4. The results from Experiment 3 (longitudinal stripe pattern) were excluded from this analysis because the number of data points in each flight were insufficient (due to the high ground speeds in this experiment) to permit an accurate frequency analysis.

Fourier transform analyses using Matlab (The Mathworks Inc) were performed on the lateral velocity values obtained from each individual flight. Observations of the individual power spectra for each flight indicated that, in almost all flights, the lateral oscillations of the honeybees contained a single, dominant frequency. The dominant frequency for each flight was obtained by identifying the frequency value with the

highest power. These values were then averaged across flights to obtain the mean frequency value for each pattern condition.

The maximum lateral velocity or amplitude of each oscillation within individual flights was identified by locating the value of lateral velocity where the preceding and succeeding values were smaller, indicating a maximum. A Gaussian filter was used to remove the noise (caused by tracking inaccuracies) from the lateral velocity data prior to the analysis to ensure accurate identification of the maximum velocity for each lateral oscillation. The mean and standard deviations of all the maximum lateral velocity values were then calculated for each flight.

Image analysis

The flights of honeybees to the feeder were filmed in three-dimensions at a rate of 100 frames per second using two synchronised CMOS cameras (MotionPro 10k, Redlake Inc). The optical axes of the cameras were oriented roughly orthogonal to each other such that one camera provided a top-view of the honeybees' flight trajectories along the tunnel, whilst the second camera provided an end-on view along the length of the tunnel. Flights were recorded in the mid-section of the tunnel over a distance of 40 cm. Flights of the honeybees to the feeder were tracked in each camera view using an automated tracking program called 'Flytrace' that was developed by Jens Lindemann in the Department of Neurobiology at the University of Bielefeld, Germany.

Data analysis

The pixel positions of the honeybee in each camera view were converted to cm values using reference frames, from which the known size and position of objects in the tunnel were measured. From this measurement, it was possible to calculate the coefficients required for the pixel to cm conversion for each position in the tunnel. The error associated with this method of converting pixel coordinates to three-dimensional world coordinates was determined to be below 1 cm.

The mean ground speed and ground height values were calculated for each individual flight and then pooled in each condition to calculate the mean values for each condition. All values are given as the mean ± standard deviation.

Statistical analysis

The identity of individual honeybees was recorded and the covariates of time, temperature, humidity and light intensity were measured during each testing period. Detailed statistical analyses to account for the effects of these sources of variation were not developed because analyses from previous experiments (e.g. Baird et al 2005, 2006) indicated that the variation between flights for an individual honeybee is similar to the variation between flights of different honeybees. These studies have also shown that the influence of covariates (time, temperature, humidity and light intensity) on the ground speed of honeybees is negligible. Normal statistical tests were used to analyse the data in the experiments described in this chapter. One-way analysis of variance (ANOVA) tests using F-statistics and Student's t-tests were used to compare the effect of wind speed and pattern on various parameters of flight.

Results

Table 3.1 shows the values of ground speed values for five wind conditions (head winds: 1 $m.s^{-1}$ and 2 $m.s^{-1}$, tail winds: 1 $m.s^{-1}$ and 2 $m.s^{-1}$ and still air) for each of the four visual textures used in this study (cross-hatch, longitudinal stripes, transverse stripes and blank). The ground height values for each of these conditions is shown in Table 3.2.

Table 3.1 Ground speed values for each visual texture and each wind condition

	Head wind 2 $m.s^{-1}$	Head wind 1 $m.s^{-1}$	Still air 0 $m.s^{-1}$	Tail wind 1 $m.s^{-1}$	Tail wind 2 $m.s^{-1}$
	mean ± sd $cm.s^{-1}$	mean ± sd $cm.s^{-1}$	mean ± sd $cm.s^{-1}$	mean ± sd $cm.s^{-1}$	mean ± sd $cm.s^{-1}$
Cross-hatch	38 ± 12	36 ± 6	36 ± 12	35 ± 12	30 ± 7
Longitudinal stripe	163 ± 28	170 ± 22	150 ± 20	163 ± 17	167 ± 24
Transverse stripe	26 ± 7	32 ± 8	34 ± 8	29 ± 9	30 ± 10
Blank	58 ± 23	57 ± 21	71 ± 16	109 ± 17	124 ± 18

Table 3.2 Ground height values for each visual texture and each wind condition

	Head wind 2 $m.s^{-1}$	Head wind 1 $m.s^{-1}$	Still air 0 $m.s^{-1}$	Tail wind 1 $m.s^{-1}$	Tail wind 2 $m.s^{-1}$
	mean ± std $cm.s^{-1}$	mean ± std $cm.s^{-1}$	mean ± std $cm.s^{-1}$	mean ± std $cm.s^{-1}$	mean ± std $cm.s^{-1}$
Cross-hatch	9 ± 4	9 ± 5	12 ± 3	13 ± 2	14 ± 2
Longitudinal stripe	6 ± 4	6 ± 3	7 ± 3	9 ± 3	1 ± 3
Transverse stripe	12 ± 4	8 ± 5	12 ± 4	13 ± 4	12 ± 3
Blank	8 ± 4	7 ± 4	6 ± 4	8 ± 3	7 ± 3

Experiment 1: Effect of wind on flight control in the presence of lateral and axial optic flow cues

This experiment investigated the effect of head and tail winds on the ground speed and ground height control of honeybees in the presence of strong axial and lateral optic flow cues. Flights of honeybees to the feeder were recorded in still air and in head and tail winds of 1 $m.s^{-1}$ and 2 $m.s^{-1}$. The mean ground speed and ground height were recorded for each individual flight. A minimum of 22 flights were recorded for each condition.

The results are shown in Figure 3.3a. The data indicate that, in the presence of strong lateral and axial optic flow cues, ground speed is unaffected by head or tail winds of up to 2 $m.s^{-1}$ ($F_4 = 1.96$, $p = 0.1061$). Ground speed is held remarkably constant despite wind speeds that exceed 5.5 times the ground speed in still air (or airspeed). In contrast, the data show that ground height is affected by wind speed ($F_4 = 11.80$, $p < 0.0001$). The mean ground height at which honeybees fly decreases with increasing head wind and increases with increasing tail wind.

Experiment 2: Effect of wind on flight control in the absence of axial optic flow cues

This experiment investigated the effect of head and tail winds on the ground speed and ground height control of honeybees when lateral optic flow cues are present and axial optic flow cues are absent. Flights of honeybees to the feeder were recorded in still air and in head and tail winds of 1 $m.s^{-1}$ and 2 $m.s^{-1}$. The mean ground speed and ground height were recorded for each individual flight. A minimum of 21 flights were recorded for each condition.

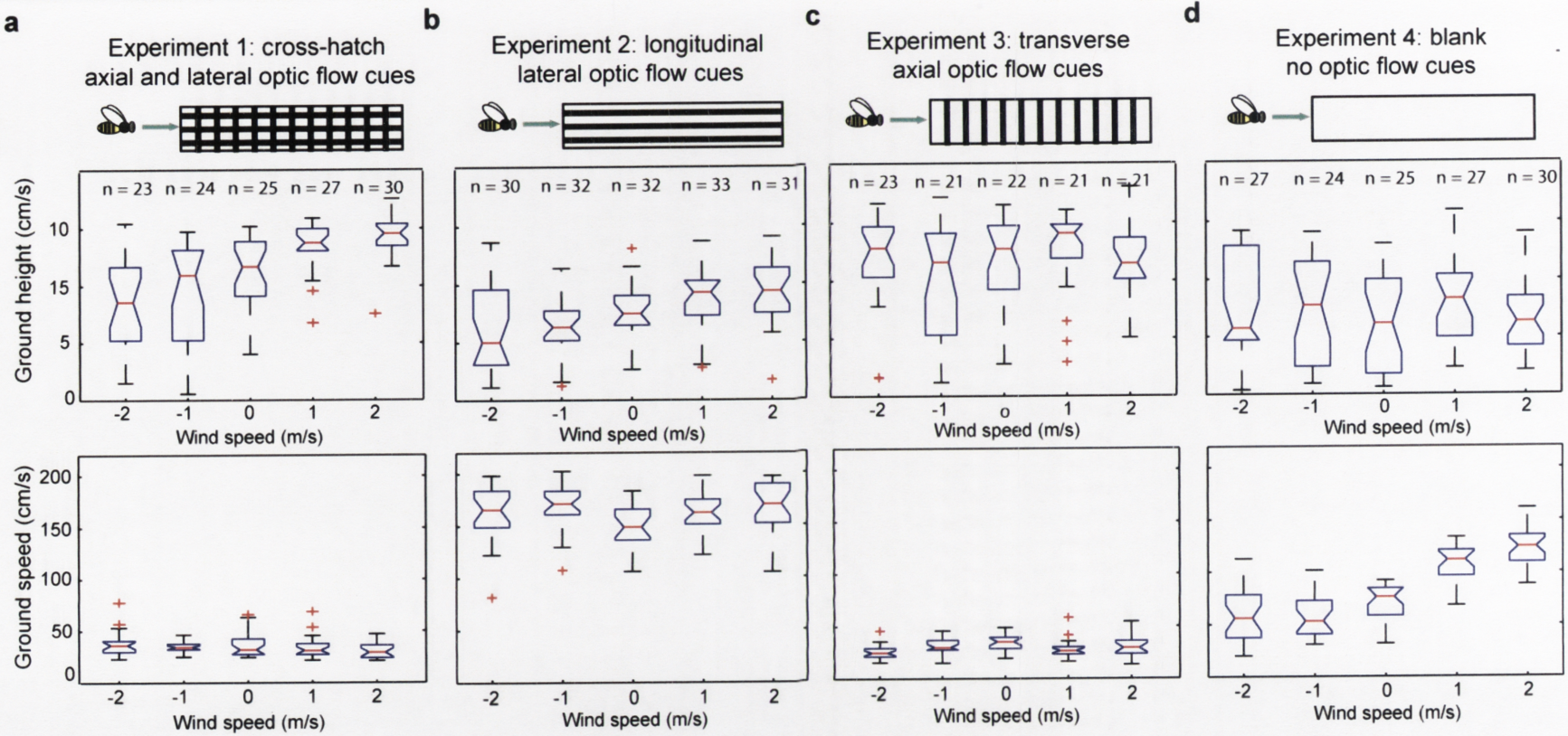

Figure 3.3 The ground speed and ground height responses of honeybees flying in wind over different visual textures
Negative wind speed values indicate head winds, positive wind speed values indicate tail winds and 0 indicates still air:
(a) Experiment 1: cross-hatch pattern
(b) Experiment 2: longitudinal stripes
(c) Experiment 3: transverse stripes
(d) Experiment 4: blank. Details of the boxplots are described in Figure 2.2.

The results are shown in Figure 3.3b. When lateral optic flow cues are present and axial optic flow cues are absent, there is a slight effect of head and tail winds on ground speed ($F_4 = 2.57$, $p = 0.0419$). In still air, the mean value of ground speed is four times faster than the value obtained in still air when strong axial and lateral optic flow cues were present (Experiment 1). The data show that ground height is affected by the speed and direction of wind ($F_4 = 5.84$, $p = 0.0003$). Ground height decreases with increasing head wind and increases with increasing tail wind.

Experiment 3: Effect of wind on flight control in the absence of lateral optic flow cues

This experiment investigated the effect of head and tail winds on the ground speed and ground height control of honeybees when lateral optic flow cues are absent and axial optic flow cues are present. Flights of honeybees to the feeder were recorded in still air and in head and tail winds of 1 $m.s^{-1}$ and 2 $m.s^{-1}$. The mean ground speed and ground height were recorded for each individual flight. A minimum of 20 flights were recorded for each condition.

The results are shown in Figure 3.3c. When lateral optic flow cues are absent and axial optic flow cues are present, there is a slight effect of head and tail winds on ground speed ($F_4 = 3.07$, $p = 0.0196$). Ground height is not affected by the speed or direction of wind ($F_4 = 1.41$, $p = 0.2356$).

Experiment 4: Effect of wind on flight control in the absence of optic flow cues

This experiment investigated the effect of head and tail winds on the ground speed and ground height control of honeybees when optic flow cues are absent. Flights of honeybees to the feeder were recorded in still air and in head and tail winds of 1 $m.s^{-1}$ and 2 $m.s^{-1}$. The mean ground speed and ground height were recorded for each individual flight. A minimum of 24 flights were recorded for each condition.

The results are shown in Figure 3.3d. When optic flow cues are absent, ground speed is affected by both head and tail winds ($F_4 = 66.41$, $p < 0.0001$). Ground speed decreased in head winds and increases in tail winds. In still air, the mean value of

ground speed is nearly twice the value obtained in Experiment 1, when strong lateral and axial optic flow cues are present. Ground height is not affected by the speed or direction of wind ($F_4 = 1$, $p = 0.4083$).

A summary of the effect of wind on ground speed and ground height for each of the visual textures is shown in Table 3.3.

Table 3.3 Summary of effects of wind on ground speed and ground height under different optic flow conditions

Experiment No. Visual texture	Optic flow cues	Effect of wind on ground speed	Effect of wind on ground height
1: Cross-hatch	Lateral and axial	Constant	Decreases in head winds Increases in tail winds
2: Longitudinal stripe	Lateral only	Near constant	Decreases in head winds Increases in tail winds
3: Transverse stripe	Axial only	Near constant	Constant
4: Blank	None	Decreases in head winds Increases in tail winds	Constant

Effect of optic flow on lateral oscillation

The data from the above experiments suggest that, in the presence of lateral optic flow cues, ground height decreases in head winds and increases in tail winds. In contrast, when lateral optic flow cues are absent, ground height is not affected by wind speed or direction. In combination with the results from the Chapter 2, this result suggests that honeybees use the lateral component of flight to control ground height.

This hypothesis was rigorously tested by comparing three key features of the lateral component of flight: the dominant frequency of lateral oscillation, the maximum lateral velocity of each oscillation (amplitude) and the within-flight variation of the value of maximum lateral speed. The effect of the presence, or absence of different components of optic flow were tested by comparing these three features of the lateral component of flight using the still air data obtained for each experiment. The results from Experiment 2 were not included as the small number of data points recorded for each flight (due to the very high ground speeds in this experiment) were insufficient for a valid analysis of the lateral component of flight.

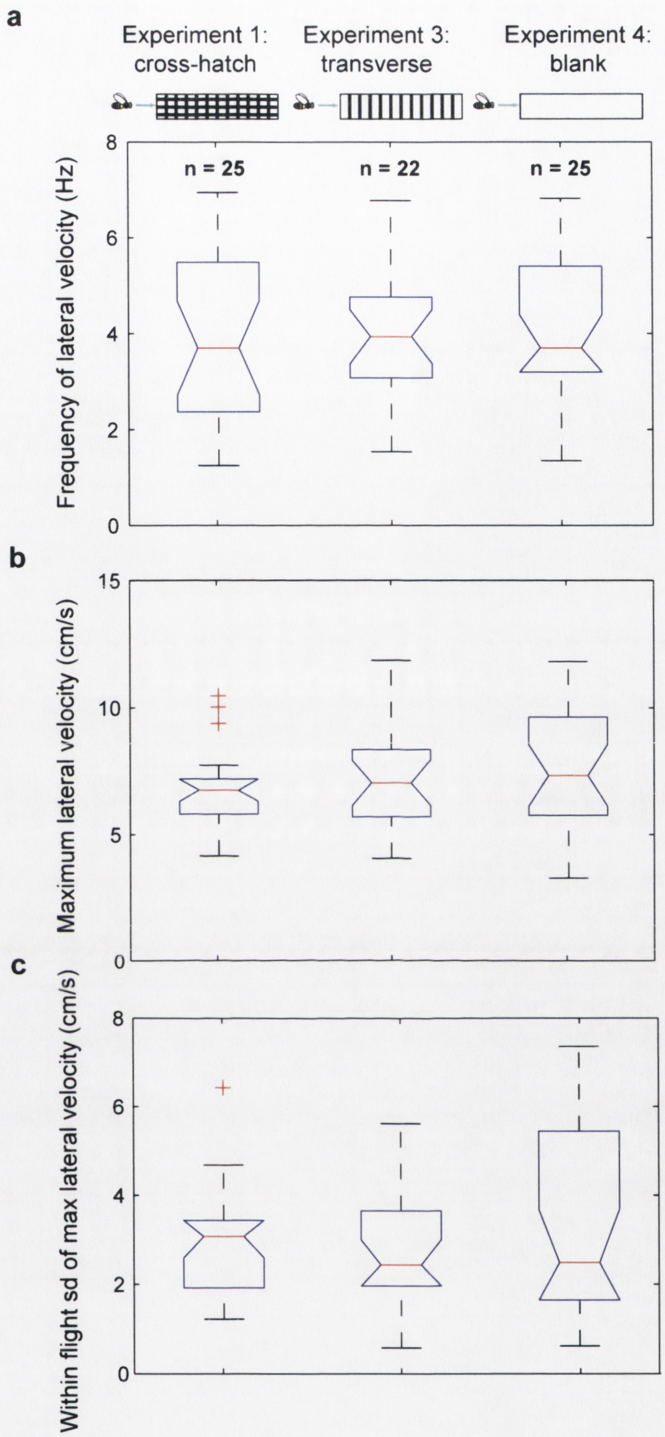

Figure 3.4 Analysis of the lateral component of flight in still air when the tunnel is lined with cross-hatch, transverse stripe and blank visual textures

(a) The frequency of lateral velocity
(b) The mean maximum lateral velocity for flights (velocity amplitude)

(c) The within-flight standard deviation of maximum lateral velocity

Figure 3.4a shows the frequency of lateral oscillation for each visual texture. The data indicate that the dominant frequency component of lateral speed is not affected by the visual texture (F_3 = 0.0173, p = 0.9828). Across conditions, the mean frequency of lateral oscillation is 4.7 ± 1.6 Hz.

Figure 3.4b shows the maximum lateral speed for each visual texture. The data indicate that the mean maximum velocity for each lateral oscillation is also unaffected by the visual texture (F_3 = 0.9668, p = 0.3854). The mean maximum lateral velocity is 7.3 ± 2.6 $cm.s^{-1}$. Figure 3.4c shows the within flight standard deviation of maximum lateral velocity values. The data indicate that the variation of maximum lateral velocity values within each flight is not affected by the visual texture in the tunnel (F_3 = 0.8504, p = 0.4317). The mean within flight standard deviation of maximum lateral velocity was 3.0 ± 1.6 $cm.s^{-1}$.

This analysis indicates that changes in the visual texture do not affect the frequency of lateral oscillation, the maximum lateral velocity or the variation of maximum lateral velocity. This suggests that the lateral component of flight is not affected by the presence or absence of lateral optic flow cues.

Discussion

Ground speed regulation in the honeybee is remarkably robust to both head and tail winds when optic flow cues are present. In the absence of optic flow cues however, honeybees do not maintain a constant ground speed. These results demonstrate that honeybees rely on optic flow cues to regulate their ground speed in wind.

The control of ground height in wind is affected by changes in visual texture. When lateral optic flow cues are present, ground height decreases in head winds and increases in tail winds. In contrast, when lateral optic flow cues are absent, ground height is unaffected by changes in wind speed and direction. Ground height is also unaffected by wind when optic flow cues are completely removed from the visual texture. In combination, these results provide compelling evidence that honeybees control their ground height using lateral optic flow cues.

Effect of wind on ground speed control

When both lateral and axial optic flow cues are present (cross-hatch), honeybees maintain a constant ground speed in both head and tail winds. Ground speed also remained constant in head and tail winds when axial optic flow cues are absent (longitudinal stripes) and when lateral optic flow cues are absent (transverse stripes). However, when all optic flow cues are removed from the visual texture (blank) ground speed is reduced in head winds and increased in tail winds – indicating that honeybees are unable to regulate their ground speed in the absence of optic flow cues.

Effect of wind on ground speed when axial optic flow cues are present

When axial optic flow cues are present (cross hatch and transverse stripes), honeybees maintain a constant ground speed in both head and tail winds. Honeybees fly at the same ground speed when both axial and lateral optic flow cues are present (cross-hatch) as they do when lateral optic flow cues are absent (transverse stripes). In still air, the mean ground speed value observed when lateral optic flow cues are absent (34.0 ± 8.4 cm.s^{-1}) is not significantly different from the mean value obtained when both lateral and axial optic flow cues are present (36.3 ± 11.8 cm.s^{-1}: $t_{46} = 0.7294$, $p = 0.4695$). Remarkably, ground speed is held constant despite head wind and tail wind speeds that were more than five times greater than the airspeed that the honeybees experienced when flying in still air. These results are consistent with the findings of previous research which indicates that, in still air (Srinivasan et al 1996; Baird et al 2005) and in head winds (Barron and Srinivasan 2006), honeybees rely on axial optic flow cues to regulate their ground speed. The results of the present study add to the results of these earlier investigations by showing for the first time that honeybees are able to regulate their ground speed in tail winds.

It is interesting to note that, when tail winds exceeded the ground speed of the honeybee (which was the case in most of the experimental conditions), the honeybees were regulating their ground speed despite flying backwards with respect to the air. When tail winds exceeded 2 m.s^{-1}, few honeybees would fly in the tunnel. Once tail winds exceeded this speed, honeybees would either land on the floor of

the tunnel or turn around and fly into the wind. This behaviour suggests that honeybees actively avoid flying in direct tail winds that are stronger than 2 $m.s^{-1}$.

Effect of wind on ground speed when axial optic flow cues are absent

When axial optic flow cues are absent and lateral optic flow cues are present (longitudinal stripes) ground speed is constant in both head and tail winds. This result conflicts with what is known about the mechanism of ground speed control – that honeybees use axial optic flow cues to regulate ground speed – because it suggests that honeybees regulate their ground speed in wind in the absence of axial optic flow cues. This remarkable result will be discussed later in this section.

The importance of optic flow cues in the regulation of ground speed becomes evident when honeybees fly in an environment where optic flow cues are absent (blank). In the absence of optic flow cues, ground speed decreases with increasing head winds and increases with increasing tail winds. The results of this experiment reveal, for the first time that, in the absence of optic flow cues, honeybees are unable to regulate their ground speed in wind.

When optic flow cues are absent, honeybees fly faster in still air (70.9 $cm.s^{-1}$) than when lateral and axial optic flow cues are present (cross-hatch: 36.3 $cm.s^{-1}$) and slower than when axial optic flow cues are absent (longitudinal stripe: 150.0 $cm.s^{1}$). This result suggests that in still air, honeybees do not regulate ground speed in the same way when optic flow cues are absent (blank) as when axial and lateral optic flow cues are present (cross-hatch) or when axial optic flow cues are absent (longitudinal stripes).

Effect of wind on ground height control

The effect of wind on ground height changes with different visual textures. Ground height is reduced in head winds and increased in tail winds when both lateral and axial optic flow cues are present (cross-hatch) and when lateral optic flow cues are present but axial optic flow cues are absent (longitudinal stripes). Ground height is not affected by head and tail winds when lateral optic flow cues are absent

(transverse stripes) and when all optic flow cues are removed from the visual texture (blank). Combined, these results suggest that honeybees use optic flow cues to control their ground height in wind.

Effect of wind on ground height when lateral optic flow cues are present

When lateral and axial optic flow cues are present (cross-hatch – Experiment 1) and when lateral optic flow cues are present and axial optic flow cues are absent (longitudinal stripes – Experiment 2), the ground height at which honeybees fly is affected by both wind speed and direction. In both of these conditions, honeybees decrease their ground height in head winds and increase their ground height in tail winds. Thus, when lateral optic flow cues are present (cross-hatch and longitudinal stripes), ground height is affected by changes in wind speed and direction.

In the presence of lateral and axial optic flow cues, honeybees change their ground height in response to head and tail winds whilst maintaining a constant ground speed. A consequence of this behaviour is that the rate of axial optic flow honeybees experience in their ventral visual field increases when they reduce their ground height in head winds and decreases when they increase their ground height in tail winds. It therefore appears that, in head and tail winds, honeybees are not holding constant the rate of axial optic flow in the ventral visual field. This result is not consistent with the predictions of the honeybee's strategy for ground speed control which indicate that honeybees would hold the rate of axial optic flow at a constant value when flying in wind.

When lateral and axial optic flow cues are present in both the lateral and ventral visual fields, it is possible that honeybees regulate their ground speed by holding constant the axial optic flow in the *lateral,* rather than the *ventral* visual field. If this is the case, then ground speed would be held constant because changes in ground height would not affect the rate of axial optic flow in the lateral visual field. It is also possible that honeybees regulate their ground speed by holding constant the overall magnitude of axial optic flow from both the lateral and ventral visual fields. In this case, the changes in the overall magnitude of axial optic flow that would have been

caused by the changes in ground height may not be large enough to elicit a detectable change in the overall magnitude of angular velocity. There is some evidence that honeybees do not elicit detectable changes in ground speed until the change in axial angular velocity exceeds a value of 78 $deg.s^{-1}$ (Baird et al 2005). It is therefore possible that the change in angular velocity over the entire visual field that would have been generated by the change in ground height in head and tail winds is not significant enough to induce a detectable change in ground speed.

Effect of wind on ground height when lateral optic flow cues are absent

When lateral optic flow cues are absent and axial optic flow cues are present (longitudinal stripes) and when optic flow cues are absent (blank), ground height remains constant in both head and tail winds. Therefore, when lateral optic flow cues are absent, ground height is not affected by wind speed or direction. This finding is consistent with the results of Chapter 2 which indicate that the ground height at which honeybees fly in the outdoor tunnel is affected only when lateral optic flow cues are removed from the visual texture.

What optic flow cues are honeybees using to control ground height in wind?

Analyses of the effect of wind on ground height have revealed two significant results: (1) ground height decreases in head winds and increases in tail winds when lateral optic flow cues are present and (2) ground height remains constant in wind when lateral optic flow cues are absent. These results could have two interpretations: that the mechanism of ground height control is operating in a 'normal' way when lateral optic flow cues are present –.reducing ground height in head winds and increasing ground height in tail winds, or that the mechanism of ground height control is operating in a 'normal' way when lateral optic flow cues are absent – holding ground height constant despite changes in wind speed and direction.

The effect of wind on ground height when lateral optic flow cues are present supports the prediction of the honeybee's ground speed control strategy: to maintain a constant rate of axial optic flow, honeybees will decrease their ground height in

head winds and increase their ground height in tail winds. This finding is also consistent with the field observations which indicate that honeybees (Wenner 1963) and bumblebees (Riley et al 1999) fly lower in head winds and higher in tail winds. The visual texture that honeybees experience in the natural environment contains strong axial and lateral optic flow cues, a situation best approximated in this study by the cross-hatch pattern. It is therefore likely that the effect of wind on ground height observed when lateral optic flow cues are present is representative of the 'normal' response of the mechanism of ground height control in wind.

It could also be argued that, by maintaining a constant ground height in wind, the mechanism of ground height control is operating in a 'normal' way when lateral optic flow cues are absent. According to this argument, honeybees are able to regulate ground height when axial optic flow cues are present and lateral optic flow cues are absent (transverse stripes); suggesting that the mechanism of ground height control is mediated by axial optic flow cues. If this were the case however, ground height should also remain constant when the visual texture is a cross-hatch pattern as this also provides strong axial optic flow cues, which it does not. Furthermore, ground height is not affected by wind when optic flow cues are absent. If the mechanism of ground height control were being mediated by optic flow cues, as the results of this study indicate, then it is unlikely that honeybees would be able to regulate ground height in the absence of optic flow cues. Consequently, the lack of effect of wind on ground height observed when lateral optic flow cues are absent is more likely to be representative of a *lack* of ground height control.

The results of this study provide evidence that in a natural visual environment, when both lateral and axial optic flow cues are present (a situation that is best approximated in this study by the cross-hatch pattern), honeybees reduce their ground height in head winds and increase their ground height in tail winds. The effect of wind on ground height is thus regulated in a 'normal' way when lateral optic flow cues are present in the visual texture (cross-hatch and longitudinal stripes). In contrast, ground height is not regulated in a 'normal' way when lateral optic flow cues are removed from the visual texture (transverse stripes and blank). These results

provide compelling evidence that ground height control in the honeybee is mediated by lateral optic flow cues.

Effect of optic flow cues on lateral oscillation

In Chapter 2, it is postulated that honeybees control their ground height by measuring some component of the lateral optic flow generated by stereotyped changes in lateral position during flight. The oscillations in lateral position that are observed over large distances in the outdoor tunnel are also evident in the small, indoor tunnel used in the current experiments. If lateral optic flow cues do play an important role in ground height control then it is necessary that these changes in lateral position are not regulated by the properties of the visual texture. If the changes in lateral position are being regulated or influenced by visual information, the optic flow cues that these lateral oscillations generate would not provide reliable information about the distance to the ground. For honeybees to be able to measure and control their ground height accurately by measuring changes in the rate of lateral optic flow, lateral oscillations must occur at a constant frequency with a constant peak velocity (amplitude).

To further test the hypothesis that honeybees use lateral optic flow cues to control ground height, a series of analyses were carried out to compare the properties of lateral oscillations under different optic flow conditions. The results from the Fourier transform analysis indicate that the frequency of lateral oscillation is not affected by the properties of the visual texture. Lateral velocity changes with the same frequency, regardless of whether the visual texture has lateral and axial optic flow cues (cross-hatch), minimal optic flow cues (blank), or when lateral optic flow cues are absent and axial optic flow cues are present (transverse stripes). An analysis of the maximum speed of lateral oscillation also indicated that the properties of the visual texture did not influence the amplitude of lateral velocity. Further analysis revealed that the within-flight variation of peak lateral velocity is also unaffected by the different conditions of optic flow. The results from these analyses provide strong evidence to support the hypothesis that changes in lateral position are not visually regulated. It is therefore possible that the lateral oscillations that are performed during flight are being used to generate lateral optic flow cues which could be then used to provide ground height information.

Effect of longitudinal stripes on flight control: an interesting case

When the tunnel is lined with longitudinal stripes, ground speed remains constant in both head and tail winds. Interestingly, the ground speed of honeybees in still air is four times higher when axial optic flow cues are absent (longitudinal stripes) than the ground speed observed when axial optic flow cues are present (cross-hatch and transverse stripes). This is consistent with the findings of previous studies which have shown that honeybees fly faster when the visual texture is composed of longitudinal stripes than when it is composed of a chequerboard pattern (Baird et al 2005; Barron et al 2006). At first glance, this finding suggests that honeybees are unable to regulate their ground speed when there are no axial optic flow cues. This leads to the prediction that, as the honeybees lack axial optic flow cues with which to measure and regulate their ground speed, ground speed would increase in tail winds and decrease in head winds. It is therefore intriguing that the ground speed regulation observed when the tunnel is lined with longitudinal stripes remains constant despite changes in wind speed and direction.

The robustness of ground speed regulation to increasing head winds in a tunnel displaying longitudinal stripes was also observed by Barron and Srinivasan (2006). The authors conclude that, in this condition, the honeybees are regulating their ground speed using optic flow cues generated by small flaws in the stripe pattern. They argue that, due to the sparseness of the optic flow cues in this condition, honeybees fly faster in order to maintain preferred rate of axial optic flow. If honeybees are in fact using sparse axial optic flow cues to regulate their ground speed when the pattern is one of longitudinal stripes, then it would predicted that they use a similar strategy to compensate for head and tail winds when optic flow cues are minimal. However, the results of Experiment 4 (blank) demonstrate that honeybees do not maintain a constant ground speed in head and tail winds when optic flow cues are absent. This result highlights the difference between the effects of removing axial optic flow cues but retaining lateral optic flow cues (longitudinal stripes) and removing all optic flow cues (blank) on ground speed regulation in the honeybee. It is evident from these results, that these two textures do not have an equivalent effect on ground speed control in the honeybee. The results from

analyses of the effect of wind on ground speed when the visual texture is one of longitudinal stripes indicates that honeybees are able to use information generated by lateral optic flow cues to maintain a constant ground speed; albeit at a higher value that when axial optic flow cues are present.

One possible method for extracting information about axial velocity is to measure the orthogonal speed of angular velocity generated by lateral oscillations over longitudinal stripes. The instantaneous velocity of the orthogonal optic flow cues would be determined by both the lateral and the axial velocity of the honeybee. Because lateral oscillations appear to be held constant, changes in the rate of orthogonal optic flow would only be caused by changes in axial velocity. Although these cues would be weak, causing an increase in ground speed, they may be sufficient to enable honeybees to regulate ground speed in wind. It is therefore possible that honeybees are able to extract sufficient information from the orthogonal optic flow cues generated by changes in lateral position to regulate their ground speed when flying over longitudinal stripes.

The ground heights at which honeybees fly in the absence of axial optic flow cues (longitudinal stripes) are lower than those observed when both lateral and axial optic flow cues are present. This is consistent with the findings of Baird et al (2006) which show that honeybees fly at a lower ground height when the floor of the tunnel displays longitudinal stripes in comparison to when the floor displays a chequerboard pattern. It is not clear why honeybees fly lower when axial optic flow cues are absent. Further investigations are necessary to understand the effect of longitudinal stripes on the mechanisms of ground speed and ground height control in the honeybee.

Conclusions

This study investigated the effects of wind on the mechanisms of visually regulated ground speed and ground height control in the honeybee. The results reveal, for the first time, details of the mechanism of ground height control in the honeybee. Honeybees appear to control their ground height in wind using lateral optic flow cues. When flying in wind or still air, honeybees change their lateral position in a near sinusoidal pattern. These oscillations in the lateral component of flight are unaffected by changes in visual texture. Overall, the findings from the present study provide strong evidence to support the theory postulated in Chapter 2 that honeybees use lateral optic flow cues to control ground height.

Chapter 4

How Honeybees Land on Vertical Surfaces

Introduction

Landing is one of the most important and difficult aspects of flight yet, despite the computational limitations of their neural architecture, insects appear to execute flawless landings with remarkable ease. To achieve smooth landings of the type that are exhibited by flying insects, it is necessary to regulate ground speed in such a way that it is close to zero at the time when the surface is contacted. This requires knowledge about the distance to the surface and the speed at which it is being approached.

The mechanisms by which flying insects regulate their ground speed when landing are not well understood. Early investigations into the landing strategies of flying insects revealed that visual cues are important for the initiation of landing responses such as the extension of the legs in preparation for contact with the target (Goodman 1960; Borst and Bahde 1986, 1988; Wehrhahn et al 1981) or the onset of deceleration in preparation for landing (Wagner 1982). Although these studies highlighted the importance of visual cues in the landing strategies of flying insects, they did not reveal how insects use these cues to regulate ground speed during landing.

Srinivasan et al (2000b) examined how honeybees make grazing landings on horizontally oriented surfaces and provided the first indication of the visual cues that honeybees use to regulate ground speed when landing. That study revealed that honeybees achieve smooth landings on horizontally oriented surfaces by holding constant the axial (front-to-back) rate of optic flow generated by the landing surface. This strategy automatically ensures that, as the insect nears the surface, the speed of approach is reduced to zero and is achieved without requiring any absolute knowledge of the distance from the surface, ground speed or the speed of descent.

This strategy of holding constant the rate of axial optic flow whilst reducing altitude ensures a smooth landing when the surface generates image motion in the axial direction. Optic flow of this type is generated when the direction of approach is parallel to the plane of the surface. The optic flow profile that an insect experiences when landing is does not necessarily contain image motion in the axial direction. The pattern of optic flow that an insect experiences as it approaches a surface can be influenced by both the angle of approach and the orientation of the surface. The optic flow profile generated on the approach to a horizontal surface is dominated by motion in the axial direction.

In contrast, the optic flow profile generated on the approach to a vertical surface is dominated by a more complex pattern of radial expansion (Koenderink and van Doorn 1976; Edwards and Ibbotson 2007). When the optic flow profile is one of radial expansion, points in the image do not move at a uniform speed or in a uniform direction. Motion in the image radiates in all directions from the centre of focus (which is determined by the direction of the insect's motion toward the surface) and at increasing speeds such that the velocity of points in the image increases from zero at the centre of focus to a maximum velocity at an angle of 45 degrees from the centre of focus. It is not clear how the landing strategy that honeybees use when making a grazing landing on a horizontal surface would function when landing on a vertical surface, as axial optic flow cues would be minimal.

How then do honeybees regulate their ground speed when landing on a vertical surface? The rate of optic flow or angular velocity generated by a surface is inversely proportional to the distance at which it is being viewed (see Srinivasan 1993 for detailed discussion). Thus, the rate of optic flow increases as the distance to the surface decreases. If the angular velocity of the image of a surface is held constant as it is being approached, the speed of the viewer will approach zero as the distance to the surface decreases. It is therefore possible that the strategy honeybees have been observed to use when approaching a horizontal surface is representative of a more general landing strategy that is concerned with holding the rate of image motion of the surface constant, irrespective of its direction. By holding constant some universal component of the optic flow generated by a surface – such as integrated value of angular velocities, the maximum angular velocity or the angular velocity at a set viewing angle – the speed of approach would automatically be adjusted to zero at the point of contact.

Image speed is given by the ratio of temporal and spatial frequency. It has been shown that those parts of the honeybee motion processing system responsible for optomotor turning responses are tuned to temporal frequency (behaviour: Kunze 1961; neurons: Ibbotson and Goodman 1990; Ibbotson 1991). Conversely, other motion sensitive cells in the honeybee visual system are known to be tuned to image speed, independent of spatial frequency (Ibbotson 2001). Therefore, a further possible strategy for regulating ground speed when landing is to hold constant the temporal frequency (the number of changes in contrast that occur per second at a set position of the retinal image), rather than the rate of optic flow generated by the landing surface. This strategy would also ensure that ground speed is reduced to zero when contact with the landing surface is made. A consequence of measuring the temporal frequency of optic flow to regulate ground speed in landing would be that the speed of approach would depend on the properties of the texture on the landing surface. In their study, Srinivasan et al (2000b) did not investigate the effect of changing the texture of the surface on the approach flights of landing honeybees. It is therefore not known whether honeybees regulate their ground speed when landing on a horizontal plane holding constant the temporal frequency rather than the angular

velocity of optic flow generated by the surface. If honeybees regulate their ground speed during landing by holding the temporal frequency, rather than the speed of the image constant, then the relationship between ground speed and distance from the surface will vary with the spatial frequency (the number of changes in contrast that occur over a set distance) of the pattern. If, on the other hand, ground speed is being regulated by holding constant the angular velocity of radial expansion (as postulated in the previous chapter), then changes in the spatial frequency of the pattern will not affect the rate of deceleration during landing.

The aim of this study is to test theoretically and empirically the hypothesis that honeybees regulate their ground speed when landing on a vertical surface by holding constant some measure of the rate of radial expansion. A mathematical model is developed to predict how ground speed would change with distance to the disc if the speed of approach to a vertical surface is being regulated by holding constant some measure of the rate of radial expansion. The hypothesis is then investigated empirically by measuring the ground speed of landing honeybees when the apparent rate of radial expansion generated by a vertically oriented spiral pattern is either increased or decreased. To investigate whether honeybees are regulating their ground speed when landing by measuring the angular velocity of radial expansion – irrespective of the spatial structure of the pattern - the ground speed of landing honeybees is measured when the spatial frequency of the spiral pattern is altered.

Model for controlling forward speed when landing on a vertical surface

This section has two main purposes. The first is to describe the properties of the spiral pattern that was used in this study. The second purpose of this section is to develop a theoretical model for the hypothesis that, when landing on a vertical surface, honeybees regulate their ground speed by holding constant some measure of the angular velocity generated by the surface. Figure 4.1 illustrates the variables that are described in the model.

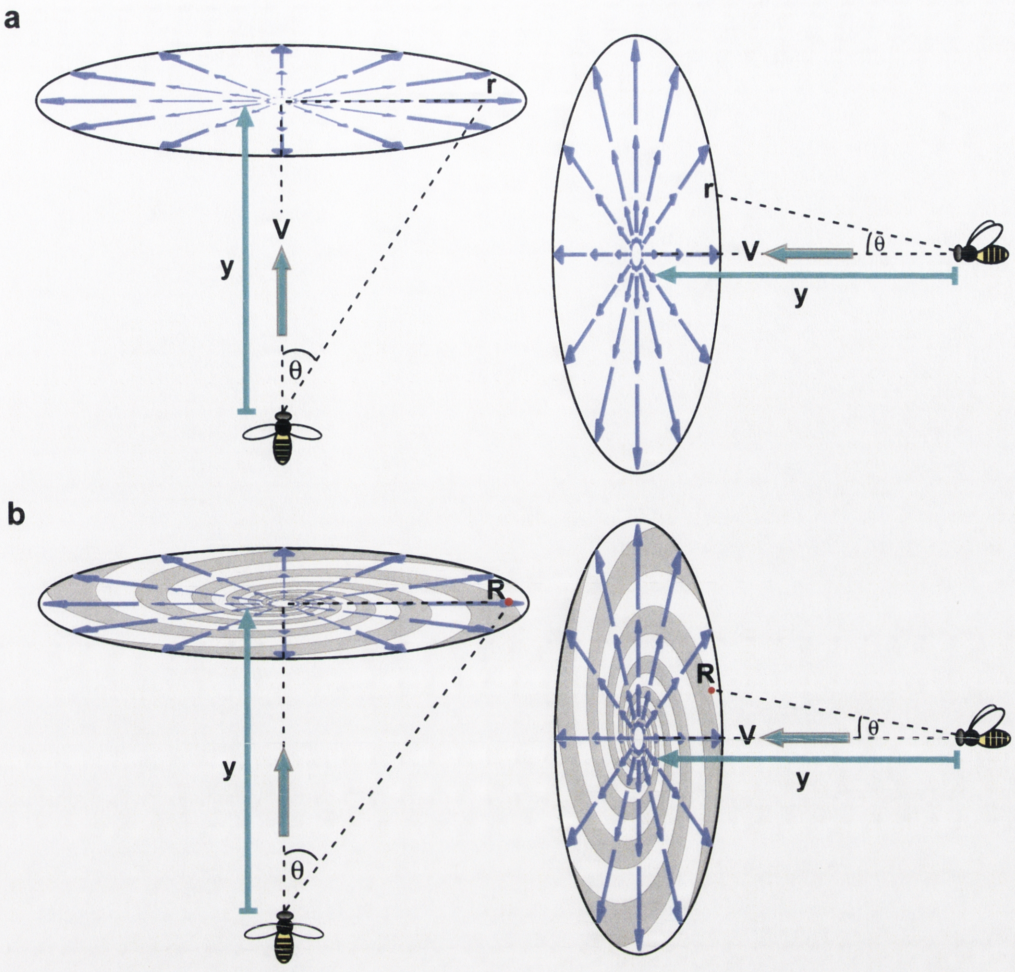

Figure 4.1 Illustration of the variables that are defined in the theoretical model for a honeybee approaching a vertically oriented disc

(a) Illustration of the model variables when a honeybee approaches a vertical surface. *V* represents the axial velocity of the honeybee approaching the disc on a horizontal trajectory, *y* is the perpendicular (axial) distance from the disc, r is a stationary point on the disc and θ is the viewing angle (the angle from the centre of focus). The blue arrows represent the pattern of radial expansion that a honeybee experiences as it approaches a vertical surface.
(b) Illustration of the model variables when a honeybee approaches a vertical disc displaying a spiral pattern. R represents a point of contrast on the arm of the spiral; this point is not stationary but changes with both spiral rotation and the distance at which it is being viewed.

What is the optic flow profile that is generated when a honeybee approaches a stationary disc?

Consider a honeybee approaching the disc along its axis at a distance of y mm with a forward motion of V mm.s^{-1} perpendicular to the plane of the disc. What is the apparent value of angular velocity of any point on the disc that will be perceived by the honeybee's eye? If we take a point on the disc that is r mm from its centre and at a viewing angle of θ rad from the position of the honeybee then:

$$\tan\theta = \frac{r}{y} \tag{1}$$

Therefore the value of r can be described as:

$$r = y \cdot \tan\theta \tag{2}$$

In the case of a honeybee approaching the stationary disc, the value of y will vary with time as the distance between the honeybee and the disc decreases but the value of r at any point on the disk will remain constant. Thus, to calculate the change in θ over time under this condition, we differentiate (1) with respect to time whilst keeping r constant:

$$\sec^2\theta \cdot \frac{d\theta}{dt} = r \cdot (-\frac{1}{y^2}) \cdot \frac{dy}{dt} \tag{3}$$

Given that:

$$V = -\frac{dy}{dt} \tag{4}$$

Substituting V for $\frac{dy}{dt}$ in (2):

$$\frac{d\theta}{dt} = \frac{Vr}{y^2} \cdot \cos^2\theta \tag{5}$$

Inserting the expression for the value of r from (2), (3) can be expressed as:

$$\frac{d\theta}{dt} = \frac{V}{y} \cdot \tan\theta \cdot \cos^2\theta \tag{6}$$

Simplifying (6), we obtain:

$$\frac{d\theta}{dt} = \frac{V}{2y} \cdot \sin 2\theta \tag{7}$$

where $\frac{d\theta}{dt}$ is the perceived angular velocity of point r as the honeybee approaches the disc. Equation (7) shows that the value of $\frac{d\theta}{dt}$ depends on the value of θ. Given that the maximum value of sin 2θ occurs at 2θ = 1.57 rad (90 deg) then, for a constant distance (y), the maximum angular velocity that a honeybee would experience as it approaches a stationary disc will be at a viewing angle of $\theta = \frac{1.57}{2} = 0.79$ rad (45 deg).

This result has two important implications. Firstly, the maximum value of angular velocity will always occur at a viewing angle (θ) of 0.79 rad (45 deg) independently of the viewing distance (y), provided that the surface that is being approached subtends an angle of more than 45 degrees on the eye. Secondly, if an approaching honeybee holds the value of angular velocity ($\frac{d\theta}{dt}$) constant for any constant viewing angle (θ), its velocity (V), will approach zero as the honeybee approaches the disc ensuring a smooth landing.

What are the properties of a spiral pattern which will generate the same optic flow profile whether it is stationary or rotating?

One way of introducing artificial changes in the optic flow that a honeybee experiences when it approaches the disc is to use a spiral stimulus which, when rotated, will either increase or decrease the overall magnitude of the optic flow profile at the contrast boundaries. Spiral patterns have been used in previous studies to generate apparent expansion or contraction experienced by an insect when viewing the pattern (e.g. Kirchner and Lengler 1994; Wicklein and Strausfeld 2000; Kelber and Zeil 1997). However, the properties of the spirals used in those studies were not discussed or described in any detail. It is therefore necessary to determine the properties that a spiral should have in order for it to produce the same optic flow profile when it is rotated, as it does when it is stationary.

To change the magnitude of the image motion experienced on the approach to a vertical surface, without changing the profile of optic flow experienced when the spiral is stationary, it is necessary to design a spiral that has specific geometric

properties. To do this, it is first important to determine how the distance between the centre of the spiral and a point of high contrast on the spiral R (i.e. a point along the edge of one of the spiral arms), changes over time when the spiral is rotated while the viewing distance (y) remains constant. To calculate the change in θ over time under this condition, we need to differentiate (1) with respect to time whilst keeping y constant:

$$\sec^2\theta \cdot \frac{d\theta}{dt} = \left(\frac{1}{y}\right) \cdot \frac{dR}{dt} \tag{8}$$

Rearranging (7) with respect to $\frac{d\theta}{dt}$ we obtain:

$$\frac{d\theta}{dt} = \left(\frac{1}{y}\right) \cdot \frac{dR}{dt} \cdot \cos^2\theta \tag{9}$$

To find the properties of the spiral that are necessary for it to produce the same optic flow profile when it is rotating as when it is stationary and being approached, it is necessary that the value of $\frac{d\theta}{dt}$ given in (5) is proportional to the value of $\frac{d\theta}{dt}$ that is given in (9). Thus:

$$\left(\frac{1}{y}\right) \cdot \frac{dR}{dt} \cdot \cos^2\theta = \alpha \cdot \frac{VR}{y^2} \cdot \cos^2\theta \tag{10}$$

where α represents the constant of proportionality.

Rewriting (10):

$$\frac{dR}{R} = \alpha . \frac{V}{y} dt \tag{11}$$

Integrating (10) with respect to time, we obtain the following expression for R:

$$R = A \cdot e^{\frac{\alpha \cdot V}{y} t} \tag{12}$$

If the spiral rotates at a constant speed, the angular velocity of ω (rad.s-1) is constant and, for a constant value of ω, can therefore be described as:

$$\psi = \omega \cdot t \tag{13}$$

So by inserting (13) into (12) we obtain:

$$R = A \cdot e^{\frac{\alpha \cdot V \cdot \psi}{\omega \cdot y}} \tag{14}$$

or $$R = A \cdot e^{B.\varphi} \tag{15}$$

where B is defined as:

$$B = \frac{\alpha \cdot .V}{\omega \cdot y} \tag{16}$$

where y, V and ω are constant.

This result demonstrates that, for a rotating spiral to generate the same optic flow profile as it does when it is static, the distance of point R from the centre of the disc must increase exponentially. This means that the radius of an arm of the spiral must increase exponentially (with a value of pitch, B) with the angle from the centre. The spiral patterns that are used in the experiments described in this study had a pitch of B = 0.3.

What is the perceived angular velocity of point R when a stationary honeybee views the rotating spiral?

From (9), the angular velocity of the rotating spiral stimulus when y is constant is:

$$\frac{d\theta}{dt} = \left(\frac{1}{y}\right) \cdot \frac{dR}{dt} \cdot \cos^2 \theta \tag{17}$$

To obtain the value of R as it changes with time, differentiate (15) with respect to time:

$$\frac{dR}{dt} = A \cdot B \cdot e^{B \cdot \psi} \cdot \frac{d\psi}{dt} = A \cdot B \cdot e^{B \cdot \psi} \cdot \omega = B \cdot R \cdot \omega = B \cdot \omega \cdot y \cdot \tan \theta \tag{18}$$

Substituting (18) into (17):

$$\frac{d\theta}{dt} = \frac{B \cdot \omega \cdot y \cdot \tan \theta}{y} \cdot \cos^2 \theta \tag{19}$$

Thus, $$\frac{d\theta}{dt} = \frac{B\omega}{2} \cdot \sin 2\theta \tag{20}$$

From (20) we see that the apparent angular rate of expansion of the spiral ($\frac{d\theta}{dt}$) will be positive if ω is positive (the spiral rotates to produce apparent expansion) and

negative if the spiral rotates in the opposite direction. At any constant viewing distance (y), the magnitude of the optic flow profile, and hence the value of angular velocity ($\frac{d\theta}{dt}$) of R, at any constant viewing angle (θ), will increase with positive values of ω and decrease for negative values of ω. Under this condition, the angular velocity of R is zero when the spiral is stationary.

What is the angular velocity at a viewing direction θ of point R when a honeybee approaches the rotating spiral pattern?

The angular velocity perceived by a honeybee at any one viewing angle as it approaches the rotating spiral can be described by adding the component of angular velocity produced when the static spiral is approached (as obtained in (8)) with the component of angular velocity generated when the spiral is rotating but the viewer's position (i.e. y) is constant (as obtained in (20)):

$$\frac{d\theta}{dt} = (\frac{V}{2y} + \frac{B\omega}{2}) \cdot \sin 2\theta \qquad (21)$$

Therefore, if the distance of the honeybee from the spiral (y), the instantaneous speed of the honeybee (V), the pitch of the spiral (B) and the angular rotation (ω) of the spiral is known then the above expression can be used to calculate the angular velocity ($\frac{d\theta}{dt}$) of the image of the spiral at the honeybee's eye at any viewing angle (θ).

From (20) it is clear that, for a given viewing angle θ, the quantity (sin2θ) is a constant (say, K).

Thus we may write

$$\frac{d\theta}{dt} = (K\frac{V}{2y} + \frac{B\omega}{2})$$

When the spiral is stationary (ω = 0), we have $\frac{d\theta}{dt} = \frac{K}{2}(\frac{V}{y})$. Therefore, if the honeybees are holding the value of $\frac{d\theta}{dt}$ constant while approaching a stationary spiral, the ratio $\frac{V}{y}$ should remain constant during the landing process. This means

that a plot of V versus y (approach speed versus distance) should be a straight line with a slope of $\frac{2}{K}\frac{d\theta}{dt}$, passing trough the origin. If the spiral is rotating at an angular velocity of ω rad/sec, then the plot of V versus y will still be a straight line, but with a slope $\frac{2}{K}\frac{d\theta}{dt} - B\omega$. This slope will be lower than the static case when the spiral expands (positive ω) and higher than the static case when the spiral contracts (negative ω). The change in the slope of the V-y relationship will depend upon the pitch of the spiral (B) and its rotational speed (ω).

If the honeybees are regulating their approach to the rotating spiral by holding some value of the perceived angular velocity constant then:

$$\frac{V_{stat}}{2y} = \left(\frac{V_{rot}}{2y} + \frac{B\omega}{2}\right) \qquad (22)$$

The honeybees are holding some value of angular velocity constant if the value of the slope of the relationship between V and y when the pattern is rotating, V_{rot}, is equal to the value of the slope when the pattern is stationary, V_{stat} minus the value of $B\omega$.

Methods

The experiments were carried out in an All Weather Honeybee Flight Facility at the Australian National University. The temperature inside the facility was maintained at 24 ± 5 °C during the day. A beehive mounted on the wall of the facility supplied the honeybees (*Apis mellifera* L.) used in the experiments.

Training

The experiments took place within a mesh flight cage which the honeybees could enter via a rectangular hole that was positioned opposite to the centre of the landing apparatus at a distance of 1.2 metres. Up to 20 honeybees were trained to enter the flight cage and land in a small tube at the centre of the experimental apparatus. After landing, the honeybees crawled through the tube to a food reward at a feeder at the rear of the apparatus. Each honeybee was marked with paint for identification of individuals.

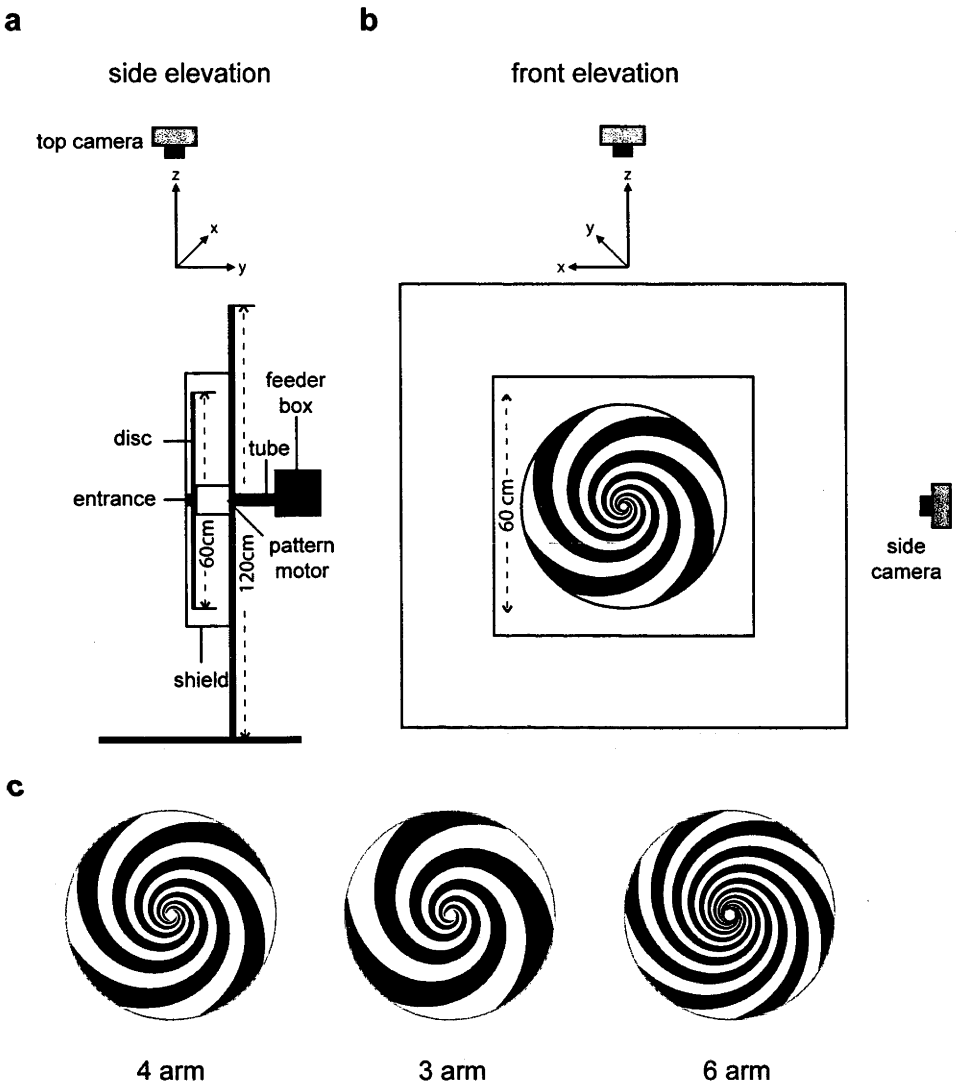

Figure 4.2 Illustration of the experimental apparatus and patterns used to investigate how honeybees land on vertical surfaces

(a) Side elevation of the apparatus
(b) Front elevation of the apparatus

(c) Spiral patterns used in Experiments 1 and 2. The 4 arm spiral was used in Experiment 1. All three spirals were used in Experiment 2 to test the effect of spatial frequency on ground speed control during landing.

Experimental Apparatus

A diagram of the experimental apparatus is shown in Figure 4.2. The apparatus consisted of a vertically oriented white Perspex disc, 60 cm in diameter. The disc was attached to a variable speed DC motor which was operated remotely by an electronic controller. The motor allowed the disc to be rotated either clockwise or anti-clockwise at a rate of up to two rotations per second (rps). A small plastic tube, 1.5 cm in diameter, was positioned at the centre of the disc for the honeybees to land on. The tube ran through the centre of the motor to the back of the apparatus where it was attached to a small black Perspex box containing a sugar-water feeder. The disc was covered from the front with a transparent Perspex shield which prevented any air currents that might be generated by the motion of the disc and allowed the honeybees to land on the apparatus when the disc was rotating.

Experiment 1: Effect of radial expansion on landing

The effect of changes in the speed of radial expansion cues on the landing response of honeybees is investigated using a black and white spiral pattern, 55 cm in diameter. The black and white areas of the spiral were equal in size and number (4 black arms and 4 white arms, as shown in Figure 4.2c). The properties of the spiral were such that the radius of each contrast border, or arm, increased exponentially with the angle from the centre with a pitch of $B = 0.3$. This ensured that the profile of optic flow generated by rotating the spiral has the same shape as that generated by the stationary spiral during rotation has the same shape as that which would be generated on the approach to the spiral when it is static.

The effect of changes in radial expansion on the ground speed of honeybees during landing was examined by recording the approaches to the spiral pattern when it was static and when it was rotating at 0.5, 1.0 and 1.5 rps either clockwise (increasing the rate of radial expansion) or anti-clockwise (decreasing the rate of radial expansion). The static spiral was used as the control condition. The honeybees were allowed to visit the static spiral for 48 hours before the experiment took place. Each 30 minute

testing period was followed by a 30 minute control period. During the control period, the honeybees continued to land on the static spiral and visit the feeder. The experimental conditions were presented in a randomised order over a period of four days with the full set of experimental conditions being presented each day.

Experiment 2: Effect of spatial frequency on landing

The effect of pattern texture on the landing responses of honeybees is investigated using three spiral patterns, each with a different number of arms. By changing the number of arms of the spiral it is possible to investigate whether the honeybees are regulating their ground speed by keeping the temporal frequency (the number of changes in contrast that occur per second at a set position of the retinal image) of the pattern constant. The properties of the spirals are the same as those of the spiral used in the Experiment 1 except that the spiral had either 3, 4 or 6 arms (Figure 4.2c). In each case, the black and white areas of the spiral are equal in size and number.

The effect of pattern texture on the ground speed of honeybees during landing was examined by recording the approaches to each spiral pattern when it was static and when it was rotating at 1.0 rps in either the clockwise (expanding) or anti-clockwise (contracting) direction. The 4-arm static spiral was used as the control condition. The honeybees were allowed to visit the static spiral for 48 hours before the experiment took place. Each 30 minute testing period was followed by a 30 minute control period. During the control period, the honeybees continued to land on the static 4-arm spiral and visit the feeder. The experimental conditions, in which both the spiral pattern and the speed and direction of pattern rotation were varied, were presented in a randomised order over a period of five days with the full set of experimental conditions being presented each day.

Recording of honeybee flight trajectories

The approach flights of honeybees to the apparatus were filmed at a rate of 250 Hz using two synchronised CMOS cameras (MotionPro 10k, Redlake Inc.). The optical axes of the cameras were positioned roughly orthogonal to each other such that one

camera provided a top-view of the honeybees' approaches to the apparatus whilst the second camera provided a side-view. Flights of the honeybees to the apparatus were tracked in each camera using an automated tracking program developed in Matlab (The Mathworks Inc.) and reconstructed in three-dimensions using the camera calibration toolbox for Matlab.

Data analysis

The speed of each honeybee was determined by calculating the change in the perpendicular distance between the honeybee and the vertical plane of the disc over time (the y axis, Figure 4.1) for each flight. Preliminary analysis revealed that the lateral component of flight velocity and the vertical component of flight velocity were much smaller in magnitude compared to that of the axial component. Given this, it follows that the axial component of flight velocity provides a good approximation of the actual magnitude of the ground speed. To normalise the individual flight data, the value of time was set to zero at the distance value that most closely corresponded to a distance of 20 cm from the landing apparatus. To analyse the change in distance over time, the flight data for each condition was averaged at every 0.004s time-step. To examine how the speed of honeybees changed with distance from the disc, the speed values from each flight were sampled at distances between 5 cm and 20 cm from the apparatus in 5 mm steps. Linear fits were applied to the speed data using the least-squares method.

Statistical analysis

Statistical models were developed to test for the influence of including multiple flight data from individual honeybees as well as the effect of covariates such as time, temperature, light intensity and humidity. The variation between flights of the same individual honeybee was found to be similar to the variation of flights of different honeybees indicating that each flight represented, in effect, an independent data point. One-way analysis of variance (ANOVA) tests using F-statistics and Student's t-tests were therefore appropriate for this data. The effects of the covariates (time,

temperature, light intensity and humidity) were tested for using a linear mixed model analysis. The covariate effects were not found to be significant and were therefore not considered in the final analyses. In Experiment 2, it was not possible to accurately record the identity of individual honeybees, so this data was not collected. However, the analyses performed in Experiment 1 showed that individual flights could effectively be treated as independent data points.

Results

Experiment 1: Effect of radial expansion on landing

Do honeybees regulate their ground speed when landing on a vertical surface by keeping some value of optic flow constant? This question was addressed by investigating the effect of altering the apparent rate of radial expansion that the honeybees experienced when landing on a vertical surface. The approach trajectories of honeybees were recorded when the disc displayed a spiral pattern that was either stationary or rotating at speeds of 0.5, 1 and 1.5 rps such that the spiral appeared to be either expanding or contracting.

Figure 4.3a shows the mean ground speed response as a function of distance for expanding spirals rotating at various speeds. Table 4.1 shows a comparison of the slopes for the linear fit for the experimental data with the slopes that are predicted by the theoretical model when the spiral is expanding. The ground speed response for each speed of spiral expansion can be approximated by linear regression models that have an intercept of zero, i.e. velocity is reduced to zero at the surface of the disc. The slopes of the linear models become lower as the speed of pattern rotation increases suggesting that, as the honeybees approach the pattern, the rate of deceleration is decreases as the perceived rate of radial expansion that is generated by the rotating spiral increases.

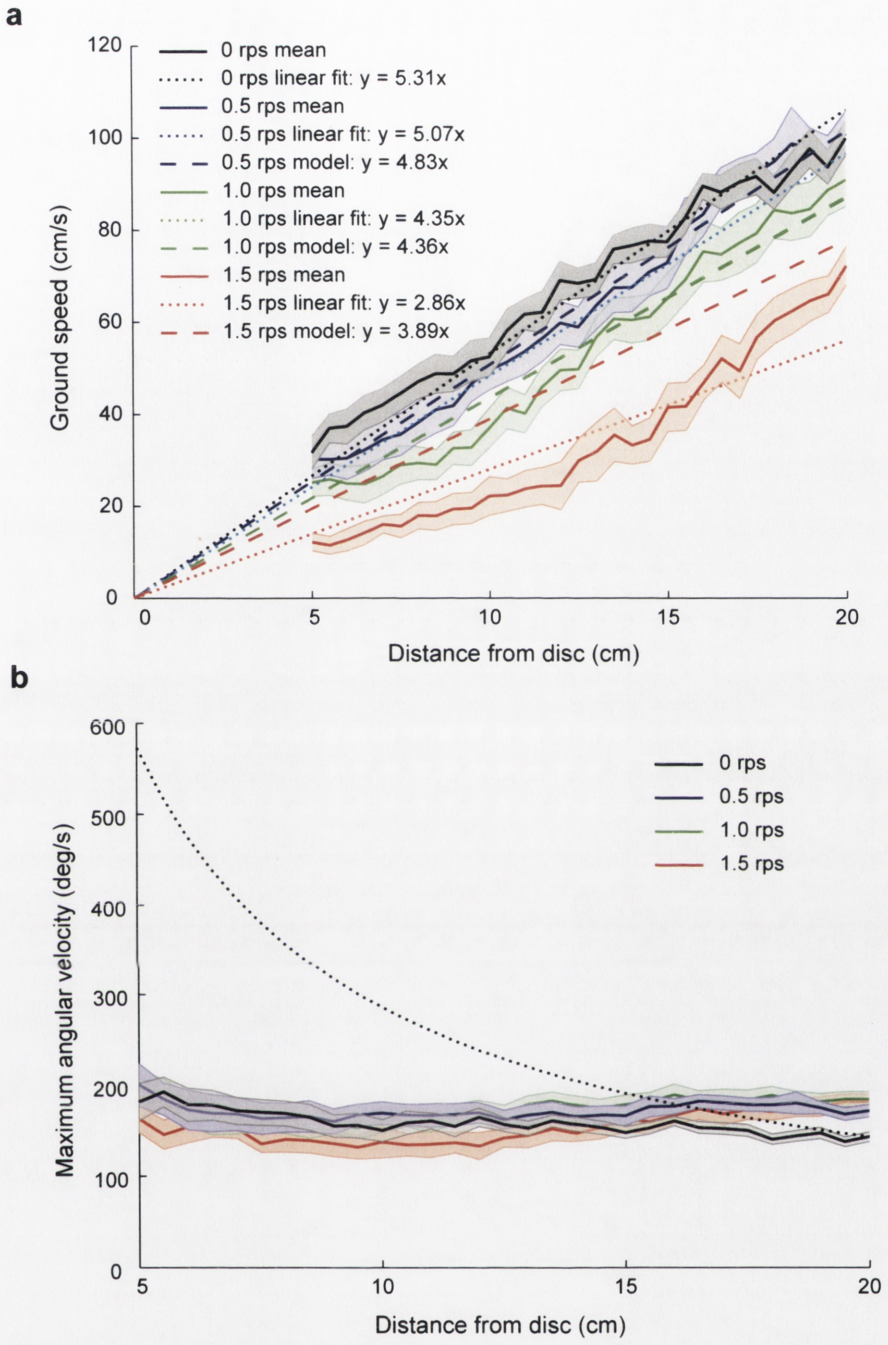

Figure 4.3 The ground speed and maximum angular velocity experienced by honeybees approaching an expanding spiral.

(a) The change in ground speed as honeybees approach the vertical disc for each speed of spiral expansion. The solid lines show the mean response for each condition, the shaded areas show the standard error of the mean for each condition. The dotted lines indicate the linear fit to the experimental data; the dashed lines indicate the model prediction of the slope. The equations for the linear fits and the model predictions are shown on the figure.
(b) The maximum angular velocity that honeybees experience as they approach the disc for each speed of spiral expansion. The black dotted line indicates the maximum angular velocity that the honeybees would experience if ground speed remained constant as the distance to the stationary disc decreased. Other details are as described in (b).

Table 4.1 Slope of the relationship between ground speed and distance from the disc from the experimental data and from the model when the spiral is expanding

	Number of flights	Number of honeybees	Slope of linear fit to experimental data	r^2	Slope predicted by model
Static: 0 rps	26	13	5.31	0.96	-
Expanding: 0.5 rps	23	9	5.07	0.99	4.83
Expanding: 1.0 rps	20	9	4.35	0.96	4.36
Expanding: 1.5 rps	20	9	2.86	0.88	3.89

From the theoretical model it is possible to predict the slope of the relationship between ground speed and distance if the honeybees are holding constant the rate of radial expansion when the spiral is expanding as when the spiral is static. These predictions are shown as dashed lines in Figure 4.3a. The relationship between ground speed and distance predicted by the model appears to approximate the observed response when the pattern is expanding at 0.5 and 1 rps. In contrast, when the spiral is expanding at 1.5 rps, the observed relationship between ground speed and distance is much lower than the response predicted by the model. These results suggest that, at low speeds of spiral expansion, the honeybees are experiencing the same rate of expansion when they approach the disc as they experience when the spiral is static. At the highest speed of pattern rotation however, the honeybees are experiencing a rate of radial expansion that is much lower than in the other conditions.

Figure 4.3b shows the maximum angular velocity (i.e. the angular velocity that would be experienced at a viewing angle of 45 degrees) that would be perceived by the honeybees as a function of distance from the disc. The maximum angular velocity that the honeybees experience as they approach the spiral does not change significantly with distance from the disc in any of the conditions. This result suggests that, when the spiral is static, or when it is expanding at 0.5 and 1.0 rps, the honeybees are adjusting their ground speed so as to keep the maximum angular velocity (or, equally, the angular velocity at any particular viewing angle) of the radial expansion of the spiral constant. There is a slight difference between the value

of angular velocity being held constant in each condition. The average maximum angular velocity that the honeybees experience as they approach the spiral decreases from 159 $deg.s^{-1}$ in the static condition to 145 $deg.s^{-1}$ at 0.5 rps and 120 $deg.s^{-1}$ at 1 rps. When the spiral is expanding at 1.5 rps, the value of maximum angular velocity, 91 $deg.s^{-1}$ is significantly lower than in the other conditions. The dotted line in Figure 4.3b indicates the theoretical curve of the maximum angular velocity that a honeybee would experience if it approached the spiral at a constant ground speed without deceleration. The difference between this theoretical curve and the maximum angular velocities that the honeybees experience for each speed of spiral expansion illustrates how well the honeybees are adjusting their ground speed to maintain a constant rate of radial expansion.

The mean ground speed response as a function of distance for contracting spirals is shown in Figure 4.4a. The slopes and intercepts of the linear fit for the relationship between ground speed and distance to the disc when the spiral is contracting is shown in Table 4.2. The ground speed response for each speed of spiral contraction can be approximated by linear regression models but, unlike when the rate of spiral expansion is increased, the intercepts do not approach zero. This indicates that, between 20 and 5 cm from the disc, the honeybees are not reducing their ground speed in such a way that it would be zero at the surface of the disc.

Table 4.2 Slope and intercept of the relationship between ground speed and distance from the disc for the experimental data when the spiral is contracting

	Number of flights	Number of honeybees	Slope of linear fit to experimental data	Intercept	r^2
Static: 0 rps	26	13	5.31	-	0.96
Contracting: 0.5 rps	26	9	3.97	381	0.96
Contracting: 1.0 rps	24	9	1.60	638	0.94
Contracting: 1.5 rps	26	10	0.63	661	0.98

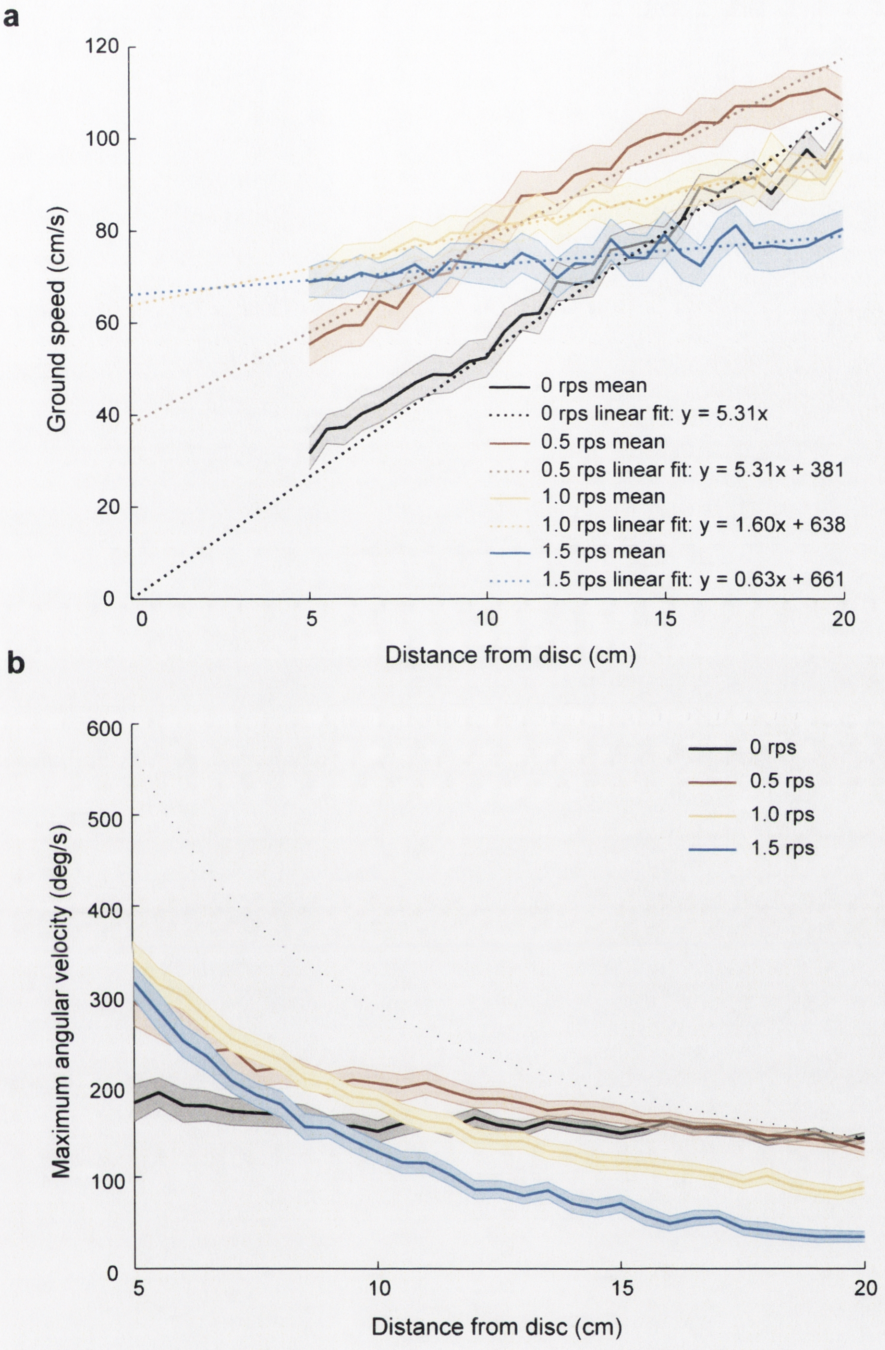

Figure 4.4 The ground speed and maximum angular velocity that the honeybees experience approaching a contracting spiral

(a) The change in ground speed as honeybees approach the disc for each speed of pattern contraction. The equations of the linear fits are shown on the figure. Other details are as described in Figure 4.3.
(b) The maximum angular velocity that the honeybees experience when they approached the disc for each speed of spiral contraction. The solid lines show the mean response for each condition, the shaded areas indicate the standard error of the mean for each condition. The black dotted line indicates the maximum angular velocity that the honeybees would experience if ground speed remained constant as the distance to the stationary disc decreased.

Figure 4.4b shows the maximum angular velocity that would be perceived by the honeybees when the spiral is contracting. In this case, the maximum angular velocity that the honeybees are experiencing increases as they approach the spiral. Between 20 and 10 cm from the disc, the honeybees are experiencing greatly reduced values of maximum angular velocity when the pattern is contracting at 1 and 1.5 rps, in comparison to the values that they experience in the static and 0.5 rps conditions. This result suggests that, at the higher speeds of pattern rotation, the honeybees are not approaching the disc at a sufficient speed to experience the same value of angular velocity that they experience when the spiral is static or rotating at 0.5 rps.

Experiment 2: Effect of pattern texture on ground speed during landing

This experiment investigates whether the ground speed of landing honeybees is affected by the spatial frequency of the pattern on the landing surface. The ground speed of honeybees approaching a vertically oriented disc was recorded when the disc displayed a spiral pattern that had 3 (low spatial frequency), 4 (medium spatial frequency) or 6 (high spatial frequency) arms. The effect of each of the spirals on ground speed was tested when the disc was stationary and when it was rotating either clockwise (increasing the rate of radial expansion) or anti-clockwise (decreasing the rate of radial expansion) at 0.5 rps. A minimum of 20 flights were analysed for each of the three spiral patterns in each of the three conditions of pattern rotation: static, expanding and contracting.

Figure 4.5 shows the relationship between ground speed and distance from the disc for the three different spirals for each condition of pattern rotation. Table 4.3 shows the slopes for the relationship between ground speed and distance to the disc for each experimental condition.

When the pattern is static, there is no significant difference between the response for the 4-arm and the 3-arm spiral ($t_{59} = 0.579$, $p = 0.5647$) or between the 3-arm and the 6-arm spiral ($t_{59} = 0.0170$, $p = 0.9865$). There is a statistically significant difference between the fits for the ground speed response for the 4-arm spiral and that of the 6-arm spiral ($t_{59} = 2.913$, $p = 0.0050$) although the difference appears to be quite small as the shapes of the responses in the figure are very similar.

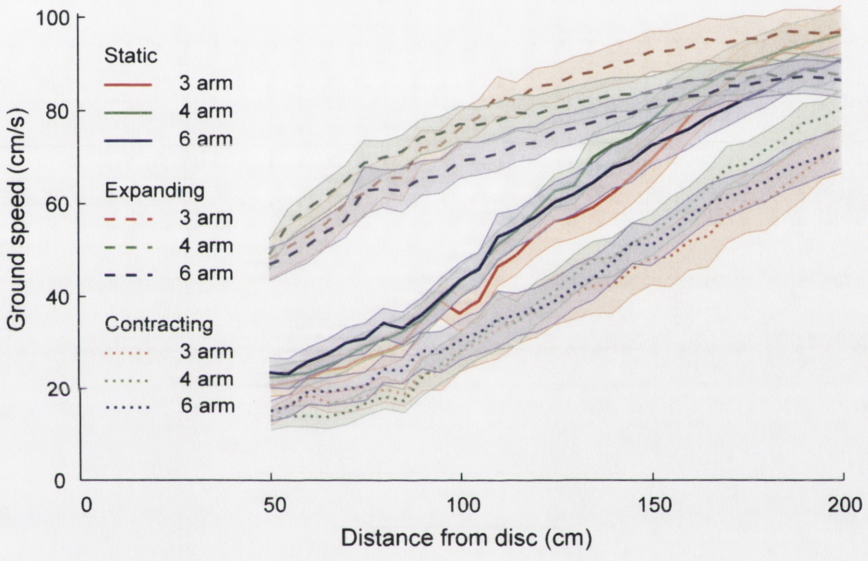

Figure 4.5 The change in ground speed over distance from the disc when the spatial frequency of the spiral is changed.

The change in ground speed over distance from the disc when the spiral has 3 arms (red data), 4 arms (green data) or 6 arms (blue data). The solid lines show the mean response when the spiral is static (solid lines), expanding at 1 rps (dashed lines) or contracting at 1 rps (dotted lines). The shaded lines indicate the standard error of the mean in each condition.

Table 4.3 Slope and intercept of the relationship between ground speed and distance from the disc for spirals with 3, 4 or 6-arms

	Number of spiral arms	Slope of linear fit to experimental data	r^2	Number of honeybees
Static 0 rps	3-arm	3.48	0.95	19
	4-arm	3.53	0.98	22
	6-arm	3.45	0.91	21
Expanding: 1.0 rps	3-arm	3.12	0.97	21
	4-arm	2.97	0.99	21
	6-arm	3.23	0.96	20
Contracting: 1.0 rps	3-arm	5.17	0.82	21
	4-arm	5.18	0.94	24
	6-arm	5.03	0.88	22

When the pattern is expanding, the relationship between ground speed and distance could also be approximated by a linear fit with an intercept of zero. When the spirals are expanding, there is some evidence of a statistically significant difference in ground speed between the 4-arm and the 6-arm spirals ($t_{50} = 2.248$, $p = 0.0283$) and between the 4-arm and the 3-arm spirals ($t_{50} = 4.245$, $p < 0.0001$). However, the shapes of the responses in Figure 5 are more similar to each other than the responses for the static and contracting conditions.

When the pattern is contracting, the relationship between ground speed and distance could not be approximated with a single linear fit between 20 cm and 5 cm from the disc. In this condition, a change in the relationship between ground speed and distance does appear to occur at approximately 10 cm from the surface of the disc for all spirals. The intercept of the slope that occurs between 10 cm and 5 cm can be described with an intercept of zero. When the spirals are contracting, there is some evidence for a statistically significant difference between the ground speed response over distance between the 4-arm and the 3-arm spirals ($t_{59} = 1.91$, $p = 0.0606$) and the 4-arm and the 6-arm spirals ($t_{59} = 3.28$, $p = 0.0017$). However, the figure shows that the shapes of the ground speed responses to contraction for each of the spiral patterns are quite similar.

Overall, the results from this experiment suggest that changes in spatial texture do not affect how honeybees control their ground speed when landing on a vertical surface. The results also indicate that there is little evidence that the spatial texture of the spiral affects the ground speed response to increases or decreases in the rate of radial expansion.

Observations of the flight trajectories

Figure 6a-c shows examples of flight trajectories of honeybees approaching the static spiral. Both the lateral and the vertical positions changed during the flight. The lateral position of the honeybees oscillated from side to side with relative constancy (Figure 4.6: a(i) – c(i)). When the velocity of these lateral translations is plotted against time, the regularity with which position changes along this axis becomes evident (Figure 4.6: a(ii) – c(ii)). These regular fluctuations are observed in many flights across all conditions. The lateral velocity in most flights contained one dominant frequency component (Figure 4.6: a(iii) – c(iii)). The vertical position of the honeybees is not always held constant but the deviations did not change in a regular pattern (Figure 4.6: a(iv) – c(iv)). The relationship between vertical velocity and time contains some small oscillations but they are not as regular or consistent as those along the lateral axis (Figure 4.6: a(v) – c(v)). A Fourier analysis was used to test whether the changes in lateral velocity observed in each flight occurred at a constant frequency (i.e. contained a single dominant frequency component), as the individual flight data suggested. The results are shown in Figure 4.7a. The results of this analysis revealed that the changes in lateral position in the static condition occurred at a mean frequency of 4.0 ± 1.6 Hz. The frequency of lateral oscillation is affected by the rotation of the spiral only when it is expanding at 1.0 and 1.5 rps. The mean maximum lateral velocity (Figure 4.7b) increased from the static value of 10.5 ± 3.2 $cm.s^{-1}$ when the spiral is rotating but appeared to be unaffected by the speed or direction of rotation.

Observations from the flight trajectories of honeybees landing on a vertical surface suggest that the lateral and vertical components of flight do not remain constant. In addition, the results of an analysis of the properties of the lateral component of flight reveal that changes in lateral velocity occur in a regular way.

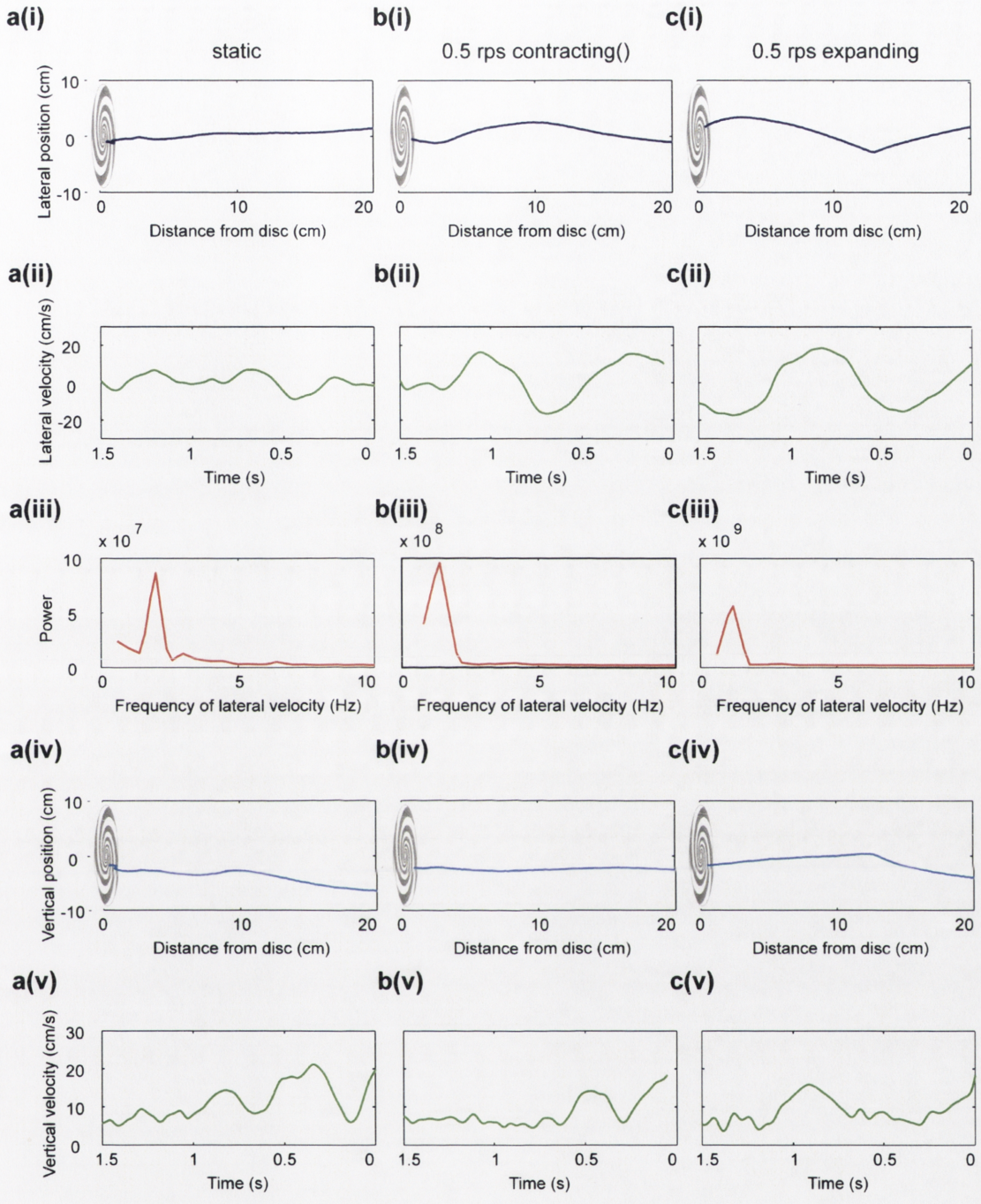

Figure 4.6 Examples of flight trajectories of honeybees landing on a vertical surface

Examples of flight trajectories when the spiral is static (a(i) – (v)), contracting at 0.5 rps (b(i) – (v)) and expanding at 0.5 rps (c(i) – (v)).
(i) Lateral position over distance from the disc
(ii) Lateral velocity over time
(iii) Power spectrum of the dominant frequency components of lateral velocity
(iv) Vertical position as distance from the disc decreases
(v) The vertical velocity over time

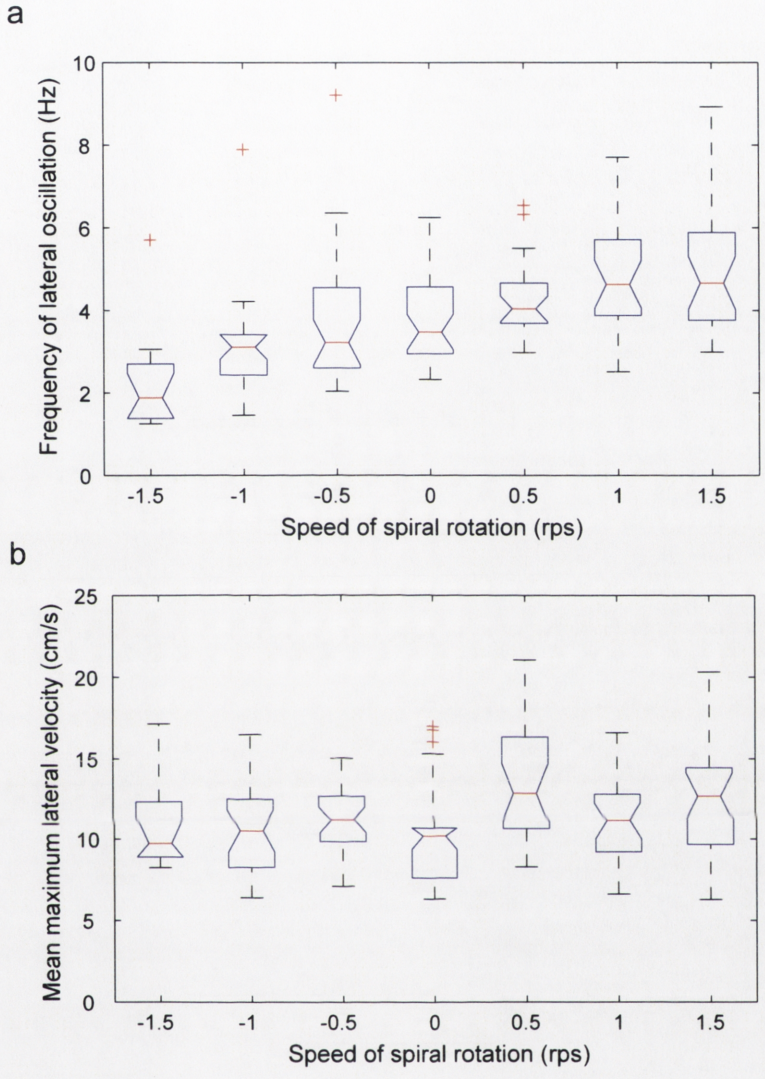

Figure 4.7 Analysis of the lateral component of the flight trajectories of honeybees landing on a vertical surface

(a) The mean dominant frequency component for each speed of spiral rotation: negative values indicate speeds when the spiral is expanding and positive values indicate speeds when the spiral is contracting.
(b) The mean maximum lateral velocity of flights approaching the spiral in all conditions. The details for the boxplot are as described in Figure 2.2.

Discussion

This study tested the hypothesis that honeybees regulate their ground speed when landing on a vertical surface by keeping constant some measure of the pattern of radial expansion generated by the surface. If honeybees do employ this strategy, then the theoretical model of this hypothesis predicts that the relationship between ground speed and distance will take a linear form with an intercept of zero, i.e. ground speed is reduced to zero at the surface of the disc. When the spiral is static or when the rate of radial expansion is increased, the relationship between ground speed and distance is largely consistent with the relationship predicted by the theoretical model. The study also found that changes in the spatial frequency of the spiral cause only minor changes in the relationship between ground speed and distance. This suggests honeybees measure the angular velocity independently of the temporal frequency of the surface when landing.

The results of this study support the hypothesis that, when landing on a vertical surface, honeybees regulate their ground speed by keeping constant the rate of optic flow generated by the surface.

What is the rate of radial expansion that the honeybees experience when approaching the spiral pattern?

The theoretical model predicts that the slope of the change in ground speed over distance will decrease as the rate of expansion increases. This is what is observed when the honeybees approach the expanding spiral. As the apparent rate of radial expansion increases, the initial ground speed of the honeybees and the change in ground speed over distance decreases. From the model, it is possible to calculate the change in ground speed that would be required for each speed of pattern rotation if the honeybees are regulating their ground speed by holding some value of the rate of radial expansion constant. In the static, 0.5 rps and 1 rps conditions, the observed change in ground speed is similar to the slope which would be required if the honeybees are holding some measure of the rate of radial expansion constant. Thus, in the static and expanding conditions, the honeybees appeared to be adjusting

their ground speed so as to keep the rate of radial expansion of the spiral at a constant value. At the highest speed of pattern rotation (1.5 rps), the observed relationship between ground speed and distance from the disc is much smaller than the relationship predicted by the model. This suggests that the honeybees are experiencing a reduced rate of radial expansion in comparison to when the spiral is rotating at slower speeds, or is static.

It is not possible to determine the precise component of the radial expansion profile that the honeybees are holding constant, as each potential measure would elicit the same change in ground speed. However, one measure of the pattern of radial expansion that can be calculated is the angular velocity that the honeybees would perceive at any given viewing angle as they approached the rotating spiral. This value of angular velocity can be calculated from the known angular speed of the spiral expansion, the distance of the honeybee from the spiral and the forward speed of the honeybee at that distance (for details see the model section). Due to the profile of image motion that is perceived when a vertical surface is approached, the maximum angular velocity in degrees per second would be experienced at a viewing angle of 45 degrees. Interestingly, the maximum angular velocity that the honeybees experienced as they approached the rotating spiral patterns did not differ significantly from the value being held constant when the spiral is stationary (190 ± 20 deg.s^{-1}) regardless of the speed of pattern rotation. The only exception to this is when the spiral is expanding at 1.5 rps, when the honeybees are experiencing a slightly lower maximum angular velocity of 150 deg.s^{-1}.

The similarity between the maximum angular velocities the honeybees experience as they approach the disc for each speed of spiral expansion indicates that ground speed is being adjusted in such a way that the perceived rate of radial expansion of the image of the spiral on the eye would remain constant.

By holding constant some measure of the angular velocity of radial expansion generated on the retina as a vertical surface is approached, ground speed will be reduced to near-zero when contact is made. This strategy could be used to regulate ground speed when landing on surfaces of any orientation (or for any angle of

approach) if the component of angular velocity being held constant is a general feature of the optic flow profile generated by the surface. Such general features could be the total integrated value of angular velocities (i.e. the magnitude of the optic flow profile), the maximum value of angular velocity (which would occur at different viewing angles for different surface orientations) or the value of angular velocity at a set viewing angle.

What is the effect of reducing the rate of radial expansion on ground speed during landing?

When the rate of radial expansion is reduced (i.e. when the spiral is contracting), honeybees do not decelerate in the same way as when the spiral is static or expanding. Although the relationship between ground speed and distance to the disc is linear, the intercept is higher than zero. This indicates that the honeybees are not regulating their ground speed in such a way that it would be reduced to zero at the disc surface. The relationship between ground speed and distance observed when the spiral is contracting at 0.5 and 1.0 rps indicates that the honeybees have initiated a landing response but the rate at which they are decelerating suggests they had overestimated the distance to the disc surface. Interestingly, when the spiral is contracting at 1.5 rps, the honeybees approached the disc at a nearly constant ground speed, indicating that they had not begun to decelerate in preparation for landing. These findings suggest that the decreased rate of optic flow that the honeybees experience when the spiral is contracting delay in the initiation of a landing response with the result that they are not reducing their ground speed in a manner that would allow them to land safely at the surface of the disc. The results of this experiment also raise an important question about the cues that trigger pre-landing deceleration in the honeybee. This question is outside of the scope of this study and will be addressed in future research.

What is the effect of spatial frequency on ground speed control during landing?

The findings of the first experiment, which examined the effect of spiral rotation on landing, suggest that honeybees rely on some measure of the pattern of radial expansion they experience when approaching a vertical surface to regulate their

ground speed. It is possible, however, that the honeybees were regulating their ground speed by keeping constant the temporal frequency of the spiral.

The second experiment investigated whether the system of motion detection that regulates ground speed during landing is sensitive to the apparent angular velocity of expansion of the image in the eye, independently of its spatial frequency content. This revealed that ground speed is not affected by changes in the temporal frequency of the spiral pattern. This confirms the conclusions from the first experiment, which suggests that honeybees regulate their ground speed by holding constant some component of the angular velocity profile generated when they approach a vertical surface. This finding is congruent with the results of previous investigations which have shown that the visual mechanism that mediates other honeybee flight behaviours – such as the centring response (Srinivasan et al 1991), the visual odometer (Si et al 2003) and ground speed control (Baird et al 2005) – is sensitive to the angular velocity, rather than the spatial frequency of optic flow. If the visual mechanism that mediates ground speed control during landing measures the spatial frequency of the landing surface, then the honeybee's approach to a surface would differ with the textural properties of the surface – thus, preventing a reliably smooth and safe landing. By measuring the angular velocity of the optic flow profile generated by the landing surface, it is possible for honeybees to ensure that the flight speed during landing will be regulated according to the distance between the honeybee and the surface regardless of its spatial texture.

How do honeybees know when to land?

Honeybees maintain a constant forward speed during cruising flight by holding constant the angular velocity of the image of the environment on their retina (Srinivasan et al 1996; Baird et al 2005). When a similar strategy is applied to the optic flow generated by a surface ground speed is reduced to near-zero as it is being approached. What cues do honeybees use to mediate between the cruising flight strategy and the landing strategy? There have been few investigations into the cues that initiate pre-landing deceleration in flying insects. A study by Wagner (1982) indicated that pre-landing deceleration in flies is initiated when the ratio of the angular size of the image to the rate of its expansion on the surface image reaches

a certain, critical value (otherwise known as tau). Tau, provides the insect with information about the time-to-contact with the approaching surface and therefore information about when to initiate a landing response. However, tau can only be used to compute the time-to-contact as the insect approaches the surface if ground speed is held constant. Another possible cue for the initiation of the landing response may be derived from the rate of optic flow of the landing surface. It is possible that pre-landing deceleration is initiated when the rate of optic flow generated by the landing surface reaches a threshold value. If the honeybees are using some measure of the rate of optic flow to determine when to begin deceleration, it could be expected that the distance at which deceleration begins increases with increasing rates of radial expansion.

What effect do changes in lateral position have on the optic flow profile generated during landing?

Both the theoretical model and the analysis of the ground speed data in this study assume that the flight trajectories of the honeybees are aligned with the centre of the spiral and that they do not contain significant lateral and vertical deviations. This assumption is necessary to allow for the calculation of both the theoretical model and the experimental analysis of the data in terms of the optic flow that the honeybees experienced. Preliminary analyses of the data from Experiment 1 supported this assumption by revealing that both the lateral and vertical components of the approach trajectories are much smaller in magnitude than the axial component of flight (flight perpendicular to the plane of the disc) and that they are roughly aligned with the centre of the disc.

Although the lateral and vertical components of the flight trajectories are not large enough to influence the calculation of ground speed, any deviations from a direct approach to the centre of the disc will introduce changes in the lateral component of optic flow in the frontal visual field (assuming that the honeybee looks straight ahead and does not fixate the reward tube during the approach).

Observations of individual flight trajectories reveal that, when approaching a vertical surface, the lateral component of flight changes in a systematic and near-

sinusoidal way. This observation indicates that the optic flow profile experienced by a honeybee when it approaches a vertical surface would not be one of pure radial expansion but would contain, in addition, an oscillating lateral component. A frequency analysis of the data revealed that, the lateral component of flight oscillated at 4.0 ± 1.6 Hz. Interestingly, the rate of lateral oscillation did not appear to be strongly affected by the rotation of the spiral except when it is expanding at higher speeds. The mean maximum lateral velocity (i.e. the amplitude of the lateral oscillations) also appeared to be unaffected by the changes in spiral rotation. Overall, the analysis of the lateral component of flight indicates that the oscillations in lateral velocity that are observed when the honeybees approach a vertical surface occur in a regular way and are relatively robust to changes in the rate of radial expansion. This suggests that the honeybees are not relying on optic flow information from the frontal visual field to regulate the changes in lateral position.

What is the consequence of oscillating the lateral component of flight during landing?

The strategy of holding constant some measure of the rate of optic flow generated by the landing surface will not provide a landing honeybee with information about the absolute distance to the surface. By adjusting ground speed according to the rate of image motion, the motion parallax cues that are generated by translation towards the target surface are effectively removed from the pattern of optic flow. Because honeybees do not appear to be using visual information to regulate their changes in lateral position, it is possible that lateral oscillations provide honeybees with cues about the absolute distance to the landing surface. Information about the distance to a landing surface could be obtained by measuring the changes in the lateral component of optic flow generated by the surface as it is approached. With regular oscillations in lateral velocity, changes in the lateral component of optic flow that are generated by these oscillations will increase as the honeybee approaches the surface.

Honeybees may also be using information generated by systematic changes in lateral position to gauge the relative size of the landing surface. Information about the relative distance to surfaces can also be extracted from the motion parallax cues that

are generated during translation. When a viewer moves the position of their eyes, nearer surfaces will generate higher image velocities than surfaces in the immediate background. The difference in the angular velocities can then be used to distinguish nearby objects from the background. Evidence that freely flying insects are able to discriminate objects from the background using motion parallax cues has been provided by investigations on honeybees (Lehrer et al 1988), moths (Pfaff and Varju 1991) and on flies (Kimmerle et al 1996). The regular oscillations in lateral velocity that are observed in this study may not only provide honeybees with information about the absolute distance to the landing surface, but may also serve to provide information about its relative size.

Conclusion

The findings of this study reveal a simple, yet elegant strategy for accomplishing smooth landings on a vertical surface. The results described here indicate that, when landing on a vertical surface, honeybees control their ground speed by holding constant some measure of the optic flow profile generated by the surface. This strategy automatically ensures that ground speed will be reduced to near-zero as the surface is approached, allowing honeybees to achieve smooth and safe landings.

The strategy described here may also represent a general strategy that could be used to regulate ground speed when approaching surfaces of any orientation. By holding constant some general measure of the optic flow profile generated by the landing surface – the overall magnitude of angular velocity, the maximum angular velocity or the value of angular velocity at a set viewing angle – ground speed would automatically be reduced to zero at the surface, regardless of its orientation or the direction of approach.

Chapter 5

General Discussion

In a single foraging flight, a honeybee may navigate over large distances across open and often turbulent environments, avoid collisions in the cluttered spaces around bushes and trees and make repeated landings on flowers of different shapes, sizes and orientations. The findings of this thesis reveal details of the elegant and computationally efficient strategies that honeybees use in order to achieve these extraordinary feats of flight control.

Visual control of ground speed

It has been demonstrated that honeybees regulate their ground speed by holding the rate of axial optic flow constant (Srinivasan 1996; Baird et al 2005). The efficacy of this simple strategy is highlighted by some of the novel findings presented in this thesis. Honeybees are able to maintain a constant forward speed in both an outdoor (Chapter 2) and an indoor (Chapter 3) flight tunnel over short and long distances.

Detailed analyses of flight trajectories reveal for the first time that honeybees are able to sustain a constant forward speed despite modulations in the lateral and vertical components of flight. The importance of optic flow cues to ground speed control in the honeybee becomes apparent when optic flow cues are minimised. Without these cues, honeybees are unable to compensate for changes in wind speed and direction. The robustness of the honeybee's ground speed control strategy in the presence of optic flow cues is accentuated by the finding that they maintain a constant ground speed in both head and tail winds of more than four times their ground speed in still air (Chapter 3). Although it has also been shown that

honeybees can maintain a constant ground speed in head winds (Barron and Srinivasan 2006), the results of Chapter 3 provide the first evidence that honeybees are also able to maintain a constant ground speed in tail winds, even when this involves flying backwards relative to the air. Overall, the findings of this thesis serve to underscore the elegance of the honeybee's ground speed control strategy by demonstrating its effectiveness in maintaining a constant forward speed under a variety of different and challenging environmental conditions.

Visual control of ground height

What cues do honeybees use to estimate their ground height?

The mechanisms by which honeybees obtain information about their ground height are not well understood. However, the results from Chapters 2 and 3, in conjunction with the findings of Baird et al (2006) demonstrate that ground height control in the honeybee is influenced by optic flow cues. In each study, ground height was affected by changes in the optic flow cues in the ventral visual field. The importance of optic flow cues for ground height regulation becomes apparent when the visual texture contains minimal optic flow cues. When optic flow cues are minimised in both the outdoor (Chapter 2) and the indoor tunnels (Chapter 3), the ground height at which honeybees fly changes in comparison to when these cues are strong. These findings provide compelling evidence that optic flow cues play an central role in ground height regulation in the honeybee.

What is the nature of the optic flow information that honeybees use to regulate ground height? Theoretically, information about the distance to the ground could be extracted from either axial or lateral optic flow cues in the ventral visual field. For a constant forward speed, the rate of axial optic flow will increase as the height above the ground decreases. To obtain an estimate of ground height it is therefore necessary that ground speed be held constant irrespective of ground height. Because honeybees regulate their ground speed by holding constant the rate of axial optic flow, ground speed will vary with ground height. To maintain axial optic flow at a constant value, ground speed will decrease at low ground heights and increase as distances from

the ground increase. As a result, axial optic flow cues in the ventral visual field alone will not provide a honeybee with information about both its ground speed and its ground height. This argument is supported experimentally by the evidence from Chapters 2 and 3. The results of Chapter 2 reveal that, in an outdoor setting, ground height is not affected by the absence of axial optic flow cues. Instead, the ground height at which honeybees fly is only affected when both lateral and axial optic flow cues are removed from the floor of the tunnel. In Chapter 3 it is shown that, in a small indoor tunnel, the effect of wind on ground height is not affected by the presence or absence of *axial* optic flow cues, but rather by the absence of *lateral* optic flow cues. It is therefore evident from the results of this thesis that honeybees do not use the axial component of optic flow in the ventral visual field to regulate their ground height.

Another method for estimating the distance to the ground could be to extract information from the lateral component of optic flow in the ventral visual field. This is because the rate of lateral optic flow in the ventral visual field will increase as the distance to the ground decreases. Unlike the axial component of optic flow however, the lateral component of optic flow will vary with ground height independently of the axial component of ground speed.

The results from Chapters 2 and 3 indicate that honeybees rely on lateral optic flow cues in the ventral visual field to regulate their ground height. In the outdoor tunnel, ground height is affected only when lateral optic flow cues are removed from the floor of the tunnel. In the indoor flight tunnel, the effect of wind on ground height regulation changes only when the lateral optic flow cues are removed from the floor of the tunnel. In both the outdoor and the indoor experimental situations, ground height control is affected only when lateral optic flow cues are removed from the visual texture. The results from Chapters 2 and 3 provide strong evidence that lateral optic flow cues are important for ground height control in the honeybee.

'Active gaze' theory for ground height control in the honeybee

Based on the findings of this thesis, it is hypothesised that honeybees use an active gaze strategy to estimate and control their ground height. The mechanism of ground height estimation proposed here is related to an active gaze strategy employed by some insects to infer the distances to surfaces in their environment. Prior to jumping across a gap, insects such as locusts and mantids estimate the distance to the target surface by moving their heads from side to side and measuring the resultant changes in optic flow (Wallace 1959; Collett 1978; Sobel 1990; Horridge 1989; Poteser and Kral 1995). The results of the studies presented in this thesis indicate that honeybees may use a similar strategy to introduce a lateral component into the pattern of translational optic flow which, in turn, provides them with information about the distance to the ground.

Is it possible for honeybees to extract ground height information from the optic flow profile generated during lateral translations?

Estimating ground height from the lateral component of optic flow

To use lateral optic flow information for ground height regulation, a honeybee must either be able to measure information about its change in lateral position independently of visual information or change its position at a constant rate with constant amplitude. Although both strategies would enable honeybees to acquire absolute information about the distance to the ground, the latter strategy would minimise the computational requirement of the distance calculation.

As discussed in Chapter 1, the apparent angular velocity generated by a surface varies with the distance at which it is being viewed and the translational speed of the viewer. Thus, to estimate the distance to the ground from the value of angular velocity generated on the retina, an accurate measure of ground speed is required. It is unlikely that honeybees would be able to acquire a reliable estimate of ground speed in moving air, especially if they are regulate their speed by holding the rate of optic flow constant. Nevertheless, it would be possible for a honeybee to extract ground height information from the lateral component of optic flow by measuring

or regulating the change in angular velocity that occurs when modulations in lateral position are made. This is because the relationship between distance and angular velocity is also true if the change in ground speed and the resultant change in angular velocity can be measured (for further discussion, see Srinivasan 1993). Srinivasan (1993) postulated that insects could measure relative changes in airspeed using wind sensitive mechanosensory hairs and use this information, combined with information about the associated changes in angular velocity to infer the distance to surfaces. It is not clear if honeybees are able to use mechanosensory information to gain knowledge of changes in air speed. Further investigation is necessary to determine whether honeybees use airspeed changes to estimate lateral velocity modulations.

To acquire information about relative, rather than absolute distance it is not even necessary for a honeybee to have an independent measure of changes in ground speed. Instead, all that is required is a regular and consistent change in lateral velocity that is generated independently of visual input (such as that which might be produced by a non-visually mediated open-loop motor command). A regular displacement of unknown magnitude, such as that which might be induced by regular changes in roll attitude, would generate a regular change in lateral optic flow in the ventral visual field. In this case, relative decreases and increases in the overall magnitude of lateral angular velocity in the ventral visual field would respectively, indicate relative increases and decreases in ground height, thereby providing the insect with information about changes in the distance to the ground.

Observations of flights in both the outdoor and indoor tunnels over long and short distances reveal that honeybees change their lateral position during flight in a uniform way. Honeybees appear to generate a near sinusoidal lateral component of flight to introduce lateral image motion into the pattern of optic flow generated by the ground.

Honeybees change their lateral velocity at a remarkably regular rate. The lateral velocity of flight oscillates at 1.7 Hz in the outdoor tunnel, 4.7 Hz in the indoor tunnel and 4.0 Hz during landing. The amplitudes or maximum lateral velocity of the lateral oscillations (outdoor tunnel: 50.0 $cm.s^{-1}$; indoor tunnel: 7.5 $cm.s^{-1}$; landing:

10.5 $cm.s^{-1}$) are also surprisingly robust to changes in visual texture. It is not clear why there is a difference between the frequency and amplitude of lateral motion in the outdoor and indoor experimental settings. It is possible that these components of lateral translation are linked to the honeybee's thrust. In the indoor tunnel, when ground speed is ten times lower, (36 $cm.s^{-1}$) than that in the outdoor tunnel (360 $cm.s^{-1}$), the frequency of lateral oscillation is nearly three times higher than the frequency of lateral oscillation observed in the outdoor tunnel. Further investigations are required to determine the factors that influence the features of these lateral oscillations.

Analyses of the lateral component of flight indicate that the near-sinusoidal oscillations in lateral velocity that were observed in this thesis occurred at a consistent frequency and with constant amplitude, despite variations in ground speed and ground height. The robustness of the lateral oscillations to changes in visual texture that were observed in this thesis suggests that these lateral translations are being driven by an open-loop motor command that is regulated independently from the visual system. This finding indicates that honeybees employ the computationally simple technique of estimating ground height from changes in lateral optic flow in the ventral visual field by changing their lateral position in a regular way.

Minimising rotational optic flow during lateral translation

A requirement of the hypothesised active gaze mechanism for ground height estimation proposed here is that rotations in the visual field must be minimised. This is because rotations distort the pattern of translational optic flow that provides information about the distance to surfaces in the environment during translation (Koenderink 1987). Collett (1978) observed that when locusts perform peering movements to gauge the distance to objects, they accompany each sideways movement of the body with a compensatory counter-rotation of the head about the yaw axis. This counter-rotation functions to minimise rotational distortions so that the pattern of optic flow during these peering movements is one of pure lateral translation. This makes the task of extracting information about the distance to objects a relatively simple one, as the retinal velocities that are generated by the

lateral translations of the insect's head can be directly converted into distance information.

The analysis of the head position of honeybees flying in a small tunnel (Chapter 2) reveals that they stabilise their heads against the changes in flight direction that are generated by lateral translation. This result suggests that the honeybees minimise the yaw rotations in the visual field that would otherwise arise from these changes in lateral position. By orienting their gaze in the direction of axial translation and minimising the effect of rotational optic flow, honeybees would be able to extract optic flow information generated from the changes in lateral position during flight.

To reduce distortions generated by rotational optic flow in the ventral visual field it is also necessary that honeybees stabilise their heads against roll rotations. It was not possible, within the scope of the experiments presented in Chapter 2 to establish whether honeybees actually do this. Recent experiments provide evidence that honeybees stabilise their heads against roll rotations when landing (Boeddeker and Hemmi, in submission). However, further investigations are necessary to determine whether this roll stabilisation also occurs in free flight.

Detecting changes in optic flow from changes in ground height

The mechanism of ground height regulation hypothesised in this thesis also requires that the honeybee visual system be able to detect changes in lateral angular velocity arising from changes in ground height. In the outdoor tunnel (Chapter 2), the image angular velocities generated by the lateral component of flight would have been small (estimated to be around 40 $deg.s^{-1}$) when the honeybees were flying at a ground height of about 1 m. At large distances from the ground, the change in lateral angular velocity that would be induced by a change in ground height would be small. It is not known whether the visual mechanisms that mediate flight control in the honeybee are capable of detecting such small changes in angular velocity.

The apparent angular velocity generated by the ground on the retina varies with the inverse of viewing height (for discussion see Chapter 1). For a honeybee flying at a ground height of 200 cm, with a maximum lateral velocity of 50 $cm.s^{-1}$, a 10 cm decrease in ground height will generate a 14 $deg.s^{-1}$ change in angular velocity. In

contrast, at a ground height of 50 cm, a 10 cm decrease in ground height will generate a change in angular velocity of 57 deg.s^{-1}. Thus, as the distance to the ground decreases, changes in ground height generate larger changes in angular velocity. When a honeybee is flying close to the ground and the risk of collision is high, small changes in ground height will generate large changes in the rate of lateral optic flow in the ventral visual field. In contrast, at larger distances from the ground, the same change in ground height will generate smaller changes in lateral optic flow in the ventral visual field. However, changes in ground height that occur at large distances from the ground are not so potentially dangerous as those that occur near the ground. Even if the visual system of a honeybee is not able to detect small differences in angular velocity generated by changes in ground height at large distances from the ground, it may be able to detect the larger differences in angular velocity generated by these height changes when the insect is closer to the ground, where the danger of collisions is increased.

Visual information processing of lateral optic flow generated by lateral translation

To obtain information about the distance to the ground, the visual system of the honeybee would be required to extract information about the magnitude and direction of angular velocity from the optic flow generated during lateral translations. Neurons that are sensitive to motion in a particular direction have been characterised in the visual pathway of the honeybee (eg. Kaiser and Bishop 1970; deVoe et al 1982; Ibbotson 2001). Theoretically, information about ground height could be extracted from directionally selective neurons that are sensitive to lateral motion, whilst information about axial translation, which is important for ground speed regulation, could be extracted from neurons that are sensitive to motion in the axial direction. Although the visual system of the honeybee contains neurons that would be capable of detecting motion in a particular direction and measuring its speed, it is not yet known whether the visual system of the honeybee has the capacity to simultaneously extract information from both the lateral and the axial component of optic flow generated during flight.

Motion adaptation is another neural mechanism that may enable the honeybee visual system to extract information about changes in the profile of lateral optic flow generated by changes in ground height. The honeybee's strategy of ground speed control ensures that the axial component of optic flow is held constant irrespective of ground height. Motion sensitive neurons in the visual pathways of flying insects have been shown adapt to prolonged periods of stimulation, a phenomenon known as motion adaptation (e.g. Srinivasan and Dvorak 1979; Maddess and Laughlin 1985; Borst and Egelhaaf 1987; Harris et al 1999, 2000; Fairhall et al 2001; Harris and O'Carroll 2002; Kurtz et al 2000; Heitwerth et al 2005). If the neural mechanisms that mediate ground speed control in the honeybee adapt to motion in the axial direction, it is possible that changes in the profile of lateral optic flow (such as those that would be generated by changes in ground height) would stand out from the overall pattern of translational optic flow. As a result, motion adaptation may serve as a mechanism by which the visual system can detect and extract information about changes in the profile of lateral optic flow (and therefore obtain information about changes in ground height) from the overall pattern of optic flow generated during flight. The honeybee's strategy of holding constant the rate of axial optic flow to regulate ground speed may also therefore assist in enhancing the detection of variations in the lateral component of optic flow in the ventral visual field.

Functional role of the 'active gaze' theory of ground height control

Because honeybees regulate their ground speed by keeping the rate of image motion in the axial direction constant, optic flow generated by pure translation in the axial direction (forward flight) would not contain information about the distance to surfaces in any region of the visual field. According to the active gaze theory of ground height control proposed in this thesis, honeybees use optic flow cues generated from regular oscillations in lateral velocity to estimate and control their ground height. Because systematic changes in lateral velocity will introduce changes in the optic flow profile throughout the visual field, this strategy would not be limited to merely providing honeybees with information about the distance to the ground, but would provide honeybees with information about distances to surfaces in all regions of the visual field. It is postulated that the strategy of changing lateral position during

flight may therefore represent a more general mechanism for gaining information about the spatial layout of the environment.

An example of the functional role of active gaze for estimating distances to surfaces in the frontal visual field is seen when honeybees land on vertical surfaces. An analysis of the flight trajectories of honeybees reveals that they change their lateral position in a regular way when landing on a vertical surface. It is possible that honeybees use the information generated by these lateral translations to obtain information about the distance to the surface when landing.

The mechanism that would enable the estimation of distance to a surface when landing is the same as the mechanism that would allow honeybees to estimate their distance from the ground (as discussed above). The only difference between estimating the distance to the ground and estimating the distance to other surfaces is the region of the visual field in which the optic flow information is measured.

It is not possible to determine whether honeybees extract information from the lateral component of optic flow in the frontal visual field to determine the distance to a landing surface from the data presented in this thesis. However, the observation that systematic lateral oscillations are present during landing raises the possibility that honeybees use an active gaze strategy to estimate the distance to surfaces even when landing.

Role of active gaze strategy for estimating the distance to surfaces

The active gaze theory of distance estimation proposed here is based on observations of honeybee flight. The reliability of the honeybee's visual odometer provides evidence that these insects have some method of estimating and controlling their ground height during free flight. The presence of lateral oscillations during landing illustrates the potential role these systematic changes in lateral velocity play in providing distance cues to surfaces throughout the visual field. The results reported in this thesis suggest that systematic changes in lateral velocity are a universal feature of honeybee flight. Although the results presented in this thesis support the active gaze theory of ground height control and distance estimation, they do not

provide conclusive evidence that honeybees use this approach to regulate their ground height or to gain knowledge about the spatial layout of their environment. Further investigations into the nature of the lateral oscillations in a wider variety of experimental situations are required to rigorously test the active gaze theory of ground height control and distance estimation.

Visual control of landing

A general theory of landing in the honeybee

Honeybees regulate their ground speed when landing on a vertical surface by holding some measure of the rate of radial expansion constant. This finding is analogous to the finding of Srinivasan et al (2000b) that honeybees regulate their ground speed by holding some measure of the rate of axial optic flow constant when landing on a horizontal plane. The similarity between these strategies provides some indication of the features of a general landing strategy in the honeybee. It is proposed here that honeybees regulate their ground speed when landing by holding constant some universal feature of the optic flow profile generated by translation toward a surface. By holding constant a universal feature of optic flow, such as the overall magnitude of optic flow, the maximum value of optic flow or the value of optic flow at a particular viewing angle, ground speed would automatically be reduced to near-zero when contact with the surface was made. A consequence of this strategy is that it would ensure smooth landings on surfaces of any orientation without requiring prior knowledge of the absolute distance to the surface, its orientation or the speed of approach. The general strategy of landing proposed here represents an elegant and computationally simple method for ensuring that ground speed will be reduced to near zero when contact with a surface is made, regardless of its orientation.

Conclusion

The simple and effective strategies for ground speed and ground height control in free flight and landing illustrated in this thesis reveal how a simple brain can achieve all of the complex tasks necessary for autonomous flight. The ground speed and ground height of a flying agent is determined by many factors. Pilots of modern

aircraft rely on the measurement of multiple parameters including air pressure sensors, thrust, local airspeed and global position to calculate and regulate their ground speed and ground height during free flight and landing.

The honeybee appears to achieve these complex tasks using only a single measurement mechanism: image velocity. This astonishing simplicity highlights the desirability of insect-inspired strategies for the control of aircraft flight. In the design of guidance systems for autonomous aerial vehicles, there is a growing need to avoid sensors that are heavy or expensive, and which use active devices such as radar, sonar or lasers. The techniques of visual guidance that are employed by flying insects, such as those reported here, suggest relatively light, inexpensive and computationally simple ways of achieving some of the desired functions like control of ground speed, ground height, landing and obstacle detection.

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